PCT/DK 03/00635
10/529397
28 MAR 2005

REC'D 31 OCT 2003
WIPO PCT

Kongeriget Danmark

Patent application No.: PA 2002 01444

Date of filing: 27 September 2002

Applicant: (Name and address)
Carlsberg A/S
Gamle Carlsberg Vej 10
DK-2500 Valby
Denmark

Title: Spatially encoded polymer matrix

IPC: -

This is to certify that the attached documents are exact copies of the above mentioned patent application as originally filed.

Patent- og Varemærkestyrelsen
Kongeriget Danmark

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Patent- og Varemærkestyrelsen
Økonomi- og Erhvervsministeriet

22 October 2003

Bo Zillo Tidemann

PATENT- OG VAREMÆRKESTYRELSEN

Spatially encoded polymer matrix

All patent and non-patent references cited in the present patent application is hereby incorporated in their entirety.

Field of invention

The present invention relates to an encoded beaded polymer matrix for combinatorial solid phase synthesis, assaying, functional proteomics, and diagnostic use. The matrix comprises a plurality of spatially immobilised particles. The spatial immobilisation of the particles confers on each bead polymer matrix a "fingerprint" which enables identification of unique beads of polymer matrices in a population of beads of different polymer matrices. The unique identification of individual bead polymer matrices makes it possible to perform combinatorial chemistry strategies while logging individual chemical transformation.

Background of invention

The synthesis of organic molecules on solid-phase synthesis beads has experienced an explosion of interest since Merrifield's pioneering work in the peptide area several decades ago. In large part, this renaissance has been driven by the advent of combinatorial chemistry, which takes advantage of the ability to synthesize large and diverse libraries of compounds efficiently on solid support.

One inherent difficulty of producing large libraries by combinatorial chemistry is the problem of how to determine the reaction history in the form of the individual synthesis steps resulting in the synthesis of any given combinatorial library member. Without such information it is not possible to deconvolute the structure of the combinatorial library member.

When employing a large number of solid supports and a large number of synthesis steps and/or processing methods, the procedure of "deconvolution" is particularly difficult. In many practical cases, where high throughput screening and fast analysis is required, this problem is inherently associated with conventional methods for

solid-phase synthesis.

Despite the tremendous practical advantages afforded by solid-phase synthesis, few reports have appeared in which a direct determination of the on-resin chemistry has been made possible in a practical way. Examples of techniques that have been used include radiography, nanoprobe nuclear magnetic resonance, single-bead fluorescence microscopy, IR spetroscopy, and optical analysis.

Combinatorial libraries may be assembled by a number of methods including the "split-and-recombine" methods described e.g. by Furka et al. (1988, 14th Int. Congr. Biochem., Prague, Czechoslovakia 5:47; 1991, Int. J. Pept. Protein Res. 37: 487-493) and by Lam et al. (1991, Nature 354: 82-84), and reviewed by Eichler et al. (1995, Medicinal Research Reviews 15 (6): 481-496) and by Balkenhohl et al. (1996, Angew. Chem. Int. Ed. Engl. 35: 2288-2337).

The split-and-recombine synthesis method involves dividing a plurality of solid supports such as polymer beads into n equal fractions representative of the number of available "building blocks" for each step of the synthesis (e.g., 20 L-amino acids, 4 different nucleotides etc.), coupling a single respective building block to each polymer bead of a corresponding fraction, and then thoroughly mixing the polymer beads of all the fractions together. This process is repeated for a total of x cycles to produce a stochastic collection of up to N^x different compounds.

The conventional split synthesis technologies referred to above present difficulties when it is desired to detect and isolate a combinatorial library member of interest. In this regard, it is necessary to first cleave the member from its solid support before identifying the member by techniques such as mass spectroscopy or HPLC. This is time consuming and cumbersome and in some cases, cleavage is not possible.

Janda (1994, Proc. Natl. Acad. Sci. USA 91: 10779-10785) describes a method in which each synthesis step of a combinatorial library member is followed by an independent coupling of an identifier tag to a solid support. Through a series of sequential chemical steps, a sequence of identifier tags are built up in parallel with the compounds being synthesised on the solid support. When the combinatorial synthesis is complete, the sequence of operations any particular solid support has gone

through may be retraced by separately analysing the tag sequence. Accordingly, use of identifier tags in this manner provides a means whereby one can identify the building blocks sequentially added to an individual solid support during the synthesis of a member of a combinatorial library.

WO 93/06121 disclose a general stochastic method for synthesising a combinatorial compound library on solid supports from which library members may be cleaved to provide a soluble library. The identifier tag may be attached directly to a member of the library or to the solid support on which the member is synthesised. Tags such as oligonucleotides can be identified by sequencing or hybridisation. Amplification of oligonucleotide tags by PCR can be employed when only trace amounts of oligonucleotides are available for analysis. However, such identification methods are time consuming and inefficient.

US 5,721,099 discloses a process for constructing complex combinatorial chemical libraries of compounds wherein each compound is produced by a single reaction series and is bound to an individual solid support on which is bound a combination of four distinguishable identifiers which differ from one another. The combination provides a specific formula comprising a tag component capable of analysis and a linking component capable of being selectively cleaved to release the tag component. Prior to analysis of a combinatorial library, each tag component must be cleaved from the support thus creating at least one additional step which is time consuming and inefficient.

Also, the above methods all rely on parallel, orthogonal synthesis of identifier tags which adds substantially to the time taken for completion of a combinatorial synthesis and has the potential of interfering with the synthesis.

Spectrometric encoding methods have also been described in which decoding of a library member is permitted by placing a solid support directly into a spectrometer for analysis. This eliminates the need for a chemical cleavage step. For example, Geysen et al. (1996, Chem. Biol. 3: 679-688) describe a method in which isotopically varied tags are used to encode a reaction history. A mass spectrometer is used to decode the reaction history by measuring the ratiometric signal afforded by the multiply isotopically labelled tags. A disadvantage of this method is the relatively

small number of multiply isotopically labeled reagents that are commercially available.

Optical encoding techniques have also been described in which the absorption or fluorescence emission spectrum of a solid support is measured. Sebestyén et al. (1993, Pept. 1992 Proc. 22nd Eur. Pept. Symp. 63-64), Camplan et al. (1994, In Innovation and Perspectives on Solid Phase Synthesis; Epton, R., Birmingham: Mayflower, 469-472), and Egner et al. (1997, Chem. Commun. 735-736) have described the use of both chromophoric and/or fluorescent tags for bead labeling in peptide combinatorial synthesis. Although this use provides an advantage for deconvoluting the structure of a library member by determining the absorption or fluorescence emission spectrum of a bead, the encoding of a large library would require the use of many chromophores or fluorophores where spectral superimposition would be a likely drawback.

WO 95/32425 discloses the coupling on beads of (i) fluorescently labelled tags having intensities that differ by a factor of at least 2, and/or (ii) multiple different fluorescent tags that can be used in varying ratios, to encode a combinatorial library. Such beads may be used in concert with flow cytometry to construct a series of combinatorial libraries by split synthesis procedure. Although this method has advantages in relation to providing a lead structure, it is necessary to construct and analyse multiple libraries commensurate with the number of stages used for the combinatorial synthesis, which is cumbersome and time consuming.

WO 97/15390 describes a physical encoding system in which chemically inert solid particles are each labelled with a unique machine readable code. The code may be a binary code although higher codes and alphanumerics are contemplated. The code may consist of surface deformations including pits, holes, hollows, grooves or notches or any combination of these. Such deformations are applied by micromachining. Alternatively, the code may reside in the shape of the particle itself. Solid particles comprising a first phase for combinatorial synthesis and a second phase containing a machine readable code are exemplified wherein the second phase may be superimposed on, or encapsulated within, the first phase. The microscopic code on the particles may be interrogated and read using a microscope-based image capture and processing system. The machine readable code may be read "on-line"

27-09-02

between different process steps of a combinatorial synthesis thus allowing the process sequence, or audit trail, for each bead to be recorded.

Nano bar coding for bioanalysis has also been described by Keating, Natan and co-workers (Science, 2001, vol. 294, 137).

WO 00/32542 discloses high throughput screening based on carriers having distinctive codes such as electronmagnetic radiation-related compounds. Similar methods have been described by Battersby et al. (2001, Drug Discovery Today, vol. 6, no. 12 (Suppl.), S19-26); Battersby and Trau (2002, Trends in Biotechnology, vol. 20, no. 4, 167-173; Meza (2000, Drug Discovery Today, vo. 1, no. 1, 38-41), and by Farrer et al. (2002, J. Am. Chem. Soc., vol. 124, no. 9, p.1994-2003).

Many of the disadvantages of the known methods described above as well as many of the needs not met by these methods are overcome by the present invention, which, as described herein below, provides several advantages over the above-described prior art methods.

Summary of the Invention

It is a first object of the present invention to prepare libraries of specific active compounds with respect to biochemical interactions, catalysis, host-guest interactions, and preferable materials properties. This is exemplified by compounds such as e.g. polypeptides (alpha-peptides, beta-peptides, and the like), polynucleotides (DNA, RNA, LNA and PNA, including non-natural and modified nucleotides comprising non-natural or modified nucleobases and/or backbones and/or carbohydrate moieties), as well as carbohydrates and mimetics of these compound classes. The libraries are preferably bead based and offer as such several advantages over prior art libraries based on chips and similar solid supports.

The active compounds of the invention can be screened for the identification of novel ligands that interact with e.g. a receptor target of interest. As such, the active compounds can be used e.g. for identifying or further develop potential drug candidates, new catalysts and materials with novel functionalities. One important applica-

27-09-02

tion of the libraries is in the diagnostic and functional proteomics area. In the following the use is exemplified by the screening of biological targets.

For any given receptor target, the probability of successfully identifying a potent ligand through a process of randomly screening molecular repertoires will increase as the size and structural diversity of the library is also increased. The present invention now makes it possible to i) rapidly identify an individual bead in a composition of beads based on individual bead "fingerprints", and ii) immediately "deconvoluting" the sequential steps employed in the solid-phase synthesis of the biologically active compound on the individual bead in question.

In order to solve this problem the invention provides in a first aspect a beaded polymer matrix in which smaller particles in the form of granulated particles or small beads have been immobilized in a random spatial arrangement such that each bead is uniquely identified by the 3-dimensional pattern formed by the particles. The particles can be labelled particles made from the same polymer material as the base synthesis polymer or they can be composed of a different material. The pattern can be detected by a property of the particle that differs from the surrounding polymer. This difference can be achieved by fluorescence labels, colour labels or by different diffractive or reflective properties of the immobilized material.

The uniquely labelled particles can be divided into portions with recording of their identity and location and subjected to different reaction conditions accordingly. Using combinatorial methods such as "Split and recombine" synthesis it is thus possible to record the precise history of reactions for each particle and thereby the structure of the product formed for each unique bead.

It is furthermore possible after screening and isolation of active hits to identify the structure of the active beads by recording the encoding pattern of the bead and correlate the pattern with all patterns recorded during the synthesis.

It is also possible to use the tool to perform diagnostic tests with mixtures of active ligands on encoded beads and after measuring the clinical values from the beads decode the results by reading the pattern of the beads.

27-09-02

A method of recording the unique pattern of each bead is by recording the relative coordinates of the center of the particles. These can be converted into absolute and unique parameters for each bead by generating the distance matrix of inter particle distances.

The coordinates of the particles in a bead can be generated in a variety of different ways.

1. A laser or conventional light exitation of the entire bead can be combined with detection along 3 orthogonal axis with three CCD cameras and the three sets of coordinates measured in 2D X,Y; Y,Z and X,Z for each particle can be used to correlate the particles to give a unique set of parameters XYZ for each immobilized particle.

2. A principle of focal or confocal microscopy can be used to obtain a 3D representation of the bead in which the 3 coordinates are the x and y of the particle in the a particular picture while the z-coordinate is derived from the focal depth.

3. Using fluorescence labelled particles a set of two focussed alternating scanning lasers along two orthogonal axis can excite the fluorophores on a moving particle in a flowcell and the fluorescence recorded with a pmt. The coordinates are generated from the two excitation positions and the position of the bead in the fluidic stream. This bead position is measured by the time of flight of the bead as determined from extinction measurement on one of the lasers.

The methods and bead products described above can be used to identify single beads out of a very large assembly of beads by rapid decoding at any point of process time. They can furthermore be used in connection with diagnostic kits where a large mixture of beads are used in a fashion similar to that of spatial arrays of e. g. DNA or protease substrates.

27-09-02

Definitions

Beaded polymer matrix: A beaded polymer matrix is a multitude of beads of crosslinked polymer formed by beading according to principles of suspension or inverse suspension polymerization, by spray polymerization, or by droplet polymerisation.

Bioactive compound: Molecules comprising a sequence of building blocks, which includes e.g. L-amino acids, D-amino acids, or synthetic amino acids, such a beta-amino acids, as well as natural and non-natural nucleotides and polynucleotides, and carbohydrates. It will also be understood that different basis sets of building blocks may be used at successive steps in the synthesis of a compound of the invention.

Carrier: Used interchangably with a beaded polymer matrix or a granulated polymer matrix.

Code: Used interchangably with the unique nature of individually identifiable beads or granules the identification of which resides in the unique spatial distribution of a plurality of particles or vacuoles. The code for each bead or granule is unique.

Coordinates: The coordinates are relative coordinates assigned to particles in the bead

2 D-coordinates: these are coordinates of particles in a 2-D projection of the particle along one of three orthogonal axis

Encoded beaded polymer matrix: This is a beaded polymer matrix formed by polymerization of a monomer mixture comprising a dispersion of particles.

Essentially: This term signifies that a physical process often yields a result that deviates from the theoretical result expected due to inheterogeniety and incomplete control of the process.

27-09-02

Essentially monodisperse: This indicate that a slight tendency towards inhomogenous location of particles can be expected due to differences in density and aggregation phenomena.

Essentially spherical: Any spherical object for which the distance from the gravitational centre to any point on the surface of the object is in the range of from a quarter of the average distance from the gravitational centre to the surface to preferably less than four times the average distance from the gravitational centre to the surface.

Essentially the same diameter: The diameters are never identical since a gaussian distribution of bead sizes is obtained during polymerization

Fluorescently detectable: An unsaturated organic molecule, a complex, an alloy or a transition metal that is excited at one wavelength and due to electronic structure and heat emmision return to ground state with emmision of a photon at a different wavelength, which can be detected.

HYDRA: PEG-triaminoethylamine star copolymer:

Individually detectable: This refer to the separation of beads in a fluidic stream of beads that allow recording of the encoding pattern of each individual bead.

PEGA: PEG-acrylamide copolymer (may be alkylated on amide)

Photon fluorescence spectroscopy: One photon fluorescence spectroscopy, which is the same as standard fluorescence spectroscopy, is based on the facts that a molecule can be excited by a single photon, and that the excited molecule after a internal process emits a photon with a lower energy than the excitation photon. The energy (the spectrum) as well as the rate of emission is specific for the molecule in its specific environment. Two-photon excitation of fluorescence is based on the principle that two photons of longer wavelength light are simultaneously absorbed by a fluorochrome which would normally be excited by a single photon, with a shorter wavelength. The non-linear optical absorption property of two-photon excitation lim-

its the fluorochrome excitation to the point of focus.

POEPOP: Polyethyleneglycol-polyoxypropylene copolymer

Resolution: This term refer to the resolution of a detection method, in a ccd frame-grap this is defined by the number of pixels and the optics used to produce the picture, in a scanning laser detection this relates to the cross-section of a laser beam at the point of excitation.

Solid phase synthesis: Synthesis where one of several of the reactants forming the target molecule is attached to a solid support e. g. a beaded polymer

Spatial position: Position of a bead or particle in space defined by Carteslan coordinates

Spatially immobilised particles: Particles which are immobilized in a surrounding polymer matrix in such a way that the individual distances between the immobilized particles are constant in a particular solvent.

SPOCC: Polymer obtained by ring opening polymerisation of partially or fully 3-methyloxetan-3-ylmethyl alkylated PEG.

Swelling: When beads or granules or particles or vacuoles are capable of swelling, any physical measurement of the afore-mentioned, including size determinations and volume determinations, refer to measurements conducted for the swelled bead or granule or particle or vacuole. Swelling of the beads are for practical reasons measured as the volume of a packed bed of beads swollen in a specific solvent and divided by the dry weight of the beads. The unit is given as ml/g. Typical solvents are water, methanol and dichloromethane, but any suitable solvent may be chosen.

Unique distance matrix: Each bead is uniquely identified by an orientation independent distance matrix describing the relative positions of particles within the encoded bead.

27-09-02

Uniquely identifiable: The encoded beads are uniquely identifiable within the limits of statistical probability of occurrence of identical beads and resolution of identification method. With a practical resolution of 1:100 and only 4 encoding particles the probability of e. g. selecting two identical beads is 10^{-6} according to Monte-Carlo simulation. A total of ~10^{15} different beads may be encoded.

Brief Description of the Drawings

Scheme 1: The immobilisation of fluorescently labelled small PEGA-particles in a PEGA-polymer by inverse suspension polymerisation of a mixture of small labelled particles with partially acryloylated bis-amino PEG

Scheme 2: Polymers particularly useful for immobilization of encoding particles are: A) PS, B) POEPS, C) POEPOP, D) SPOCC, E) PEGA, F) CLEAR, G) Expansin, H) Polyamide, I) Jandagel or derivatives of any of these. Alginates, gelatines, aluminas, pore glasses and silicas are other types of useful supports.

Figure 1: The principle of recording of coordinates for encoding particles in a bead and conversion to a orientation independent distance matrix that uniquely identifies the single bead. Three unique distances within 500 micron beads comprising 1 micron particles there is 65.449.846 positions giving in theory ~ 3×10^{23} combinations from which at least 3×10^{15} will be unique. Orthogonal recording on three LCD detectors yields 9 co-ordinates. These can be paired based either on fluorescence intensity or on a fourth non orthogonal detector. Conversion to inter-particle distances gives an orientation independent parameter set. The 3 distances (3 particles) are sorted and indexed according to the longest distance. Beads are sorted according to number (3, 4 or 5) of particles prior to use. If beads with 4 particles are used, 6 distances are stored etc. Alternatively, two scanning lasers can directly yield the three coordinate sets on the moving bead.

Figure 2. Recording of particle coordinates with 3 ccd's for xy-plane placed along 3 orthogonal axis by excitation of the bead with a single laser pulse.

Figure 3. Recording of coordinates of particles in a bead by focal or confocal microscopy.

27-09-02

Figure 4. Recording of coordinates of particles in a moving bead by two alternating scanning lasers.

Figure 5. Recording of coordinates of particles using a single laser and a rotating mirror with reflection from 3 angles.

Scheme 3. Split and combine synthesis of a library of 400-dipeptides on 1000 beads with reading of encoding at each reaction step.

Figure 6 A-I. Selected examples of pictures of 20 selected beads shown with the pool of identification out of 20 possible for each reaction step.

Figure 7 A and B. Two sets of 3 orthogonal pictures with 3 fluorescent particles immobilised in a bead.

Figure 8. Monte Carlo simulation of 10'000 beads of 500 units diameter. Using 4 fluorescent particles, the relative frequencies for different encoding separations $\cap v$ (see text) are plotted. The main figure is given on the basis of 100'000 out of the total of $\binom{10^4}{2} \approx 50$ million pair-wise distances between the encoding vectors, while the inset uses all 50 millions. Note that only in 2 out of 10'000 cases $\cap v \geq 12$ units, and that in none of the 50 million pairs $\cap v \geq 3$ units.

Table 1. Visually identified beads in each reaction step indicating amino acid sequence and verification of the result by Edman degradation sequencing.

Detailed Description of the Invention

Polymer matrix

It is one object of the present invention to provide an encoded, beaded or granulated polymer matrix for solid phase synthesis comprising beads or granules each comprising a plurality of spatially immobilised particles or vacuoles, wherein each particle or vacuole is individually detectable. The matrix has different optical or spectro-

scopic properties from those of the immobilised particles or vacuoles. The immobilised particles or vacuoles can be monodisperse or heterodisperse, and the immobilised particles can be regular spherical beads or vacuoles, or they can be irregular particles.

Each beaded or granulated polymer matrix preferably comprises at least 2 particles, such as at least 3 particles, for example at least 4 particles. The particles can be essentially spherical, and preferably at least 2 such as 3, for example 5 of said particles have essentially the same diameter. The particles are preferably essentially monodisperse and/or less than 10 micrometer in diameter, such as less than 5 micrometer in diameter, for example less than 1 micrometer in diameter, such as less than 0.1 micrometer in diameter.

Beads and granules of the polymer matrix

The present invention resides, at least in part, in a bead on which a compound can be synthesised, wherein the bead has at least two markers integrally associated therewith, which markers are detectable and/or quantifiable during synthesis of the compound. The markers define a code identifying the bead before, during and after synthesis of a compound. Through the use of its plurality of detectable and/or quantifiable markers, preferably optically detectable and/or quantifiable markers, the bead of the present invention provides more "pre-encoded" information compared to other beads of the prior art and thus provides larger combinational library sizes that can be encoded.

This "pre-encoded" information may be read by specialized flow cytometers and can be used to track the synthetic history of an individual bead in a combinatorial process as described hereinafter. The larger the diversity of detectable and/or quantifiable markers of a bead, the greater the degree of decipherability or resolution of the bead in a large population of beads. In this regard, each detectable and/or quantifiable marker of a bead provides at least a part of the information required to distinctively identify the bead. The larger the number of such markers, the more detailed the identifying information that is compilable for a given bead, which may be used to distinguish that bead from other beads. On the other hand the complication of de-

tection is increased markedly with the number of markers.

Markers

The particles can comprise a marker, which is detectable by any form of electromagnetic radiation including fluorescent emmision. However, the marker can also be detected by fast spectroscopic techniques other than fluorescence spectroscopy. The particles of said beaded or granulated polymer matrix in one embodiment comprise a spectroscopically detectable marker and/or a fluorescently detectable marker.

Fluorescently detectable markers are preferably selected from the fluorescent group of compounds and materials consisting of fluorencent organic polycyclic compounds, conjugated vinylic compounds, heterocyclic transition metal complexes, rare earth metal compounds, inorganic oxides and glasses.

Fluorescently detectable markers can be detected by two photon fluorescence spectroscopy and/or by one photon fluorescence spectroscopy. Fluorescently detectable markers can additionally be detected by time-correlated photon fluorescence spectroscopy.

The fluorescently detectable marker is preferably selected from the group consisting of dyes based on the structure of fluoresceln, oregon green, rhodamine, aminobenzoic acid, Alexa™ probes, BODIPY-dyes, cascade blue dye, coumarine, naphthalenes, dansyl, indoles, pyrenes pyridyloxazole, cascade yellow dye, Dapoxyl Dye, Fluorescamine, aromatic ortho dialdehydes, OPA and NDA, ATTO-Tag's, 7-Nitrobenz-2-Oxa-1,3-Diazole or derivatives thereof. The fluorescently detectable marker in one embodiment is preferably a UV or visible light-excitable microsphere.

Examples of detection by fast spectroscopic techniques other than fluorescence spectroscopy include, but is not limited to fast spectroscopic techniques such as infrared spectroscopy, raman spectroscopy, visble light spectroscopy, UV spectroscopy, electron spin resonance, and nuclear magnetic resonance.

27-09-02

Also included in the present invention are markers which are detectable by fast detection techniques other than spectroscopy, such as light scattering, reflection, diffraction or light rotation.

Electromagnetic radiation-related markers are preferably selected from the group consisting of fluorescence emission, luminescence, phosphorescence, infrared radiation, electromagnetic scattering including light and X-ray scattering, light transmittance, light absorbance and electrical impedance.

Preferably, the electromagnetic radiation-related marker is a light emitting, light transmitting or light absorbing marker detectable by illuminating the particle with incident light of one or more selected wavelengths or of one or more selected vectors.

It is preferred that at least one of the markers of a bead is an electromagnetic radiation-related marker suitably selected from the group consisting of atomic or molecular fluorescence emission, luminescence, phosphorescence, infrared radiation, electromagnetic scattering including light and X-ray scattering, light transmittance, light absorbance and electrical impedance.

The fluorescence emission can result from e.g. excitation of one or more fluorescent markers attached to, or contained within, the bead. In the case of two or more fluorescent markers being utilised, the markers can be the same and the markers can comprise the same or varying amounts of a fluorophore. In the latter case the markers are intensity-differentiated.

Alternatively, the markers may be different wherein they are present in a ratio of 1: 1 or varying ratios. Reference may be made in this regard to WO 95/32425 which is incorporated herein by reference.

Exemplary fluorophores which may be used in accordance with the present invention include those listed in WO 93/06121, which is incorporated by reference herein.

Any suitable fluorescent dye may be used for incorporation into the bead of the invention. For example, reference may be made to U. S. Patents 5,573,909 (Singer et

al., which is incorporated herein by reference) and 5,326,692 (Brinkley et al., which is incorporated herein by reference) which describe a plethora of fluorescent dyes. Reference may also be made to fluorescent dyes described in U. S. Patent Nos. 5,227,487,5,274,113, 5,405,975, 5,433,896,-5,442,045, 5,451,663, 5;453,517, 5,459,276, 5,516,864,-5,648,270 and 5,723,218, which are all incorporated herein by reference.

In one embodiment, one or more of the fluorescent markers can preferably be incorporated into a microparticle, such as a polymeric microparticle, or a ceramic microparticle. Such microparticles can preferably be attached to a bead by use of e.g. colloidal interactions as for example disclosed by Trau and Bryant in PCT/AU98/00944, incorporated herein by reference.

When the marker is spectroscopically detectable, there is in one embodiment provided a marker capable of being probed by a range of frequencies differing by less than about 20%, such as less than about 10%, based on the numerical highest frequency value. The marker can also be probed by one or more predetermined frequencies.

Any suitable method of analysing fluorescence emission is encompassed by the present invention. In this regard, the invention contemplates techniques including, but not restricted to, 2-photon and 3-photon time resolved fluorescence spectroscopy as for example disclosed by Lakowicz et al. (1997, Biophys. J., 72: 567, incorporated herein by reference), fluorescence lifetime imaging as for example disclosed by Eriksson et al. (1993, Biophys. J., 2: 64, incorporated herein by reference), and fluorescence resonance energy transfer as for example disclosed by Youvan et al. (1997, Biotechnology et alia 3: 1-18).

Luminescence and phosphorescence may result respectively from a suitable luminescent or phosphorescent label as is known in the art. Any optical means of identifying such label may be used in this regard.

Infrared radiation may result from a suitable infrared dye. Exemplary infrared dyes that may be employed in the invention include, but are not restricted to, those disclosed in Lewis et al. (1999, Dyes Pigm. 42 (2): 197), Tawa et al. (1998, Mater. Res.

27-09-02

Soc. Symp. Proc. 488 (Electrical, Optical, and Magnetic Properties of Organic Solid-State Materials IV), 885-890), Daneshvar, et al. (1999, J. Immunol. Methods 226 (1-2): 119-128), Rapaport et al. (1999, Appl. Phys: Lett. 74 (3): 329-331) and Durig et al. (1993, J. Raman Spectrosc. 24 (5): 281-5), which are incorporated herein by reference. Any suitable infrared spectroscopic method may be employed to interrogate the infrared dye. For instance, fourier transform infrared spectroscopy as for example described by Rahman et al. (1998, J. Org. Chem., 63: 6196, incorporated herein by reference) may be used in this regard.

Suitably, electromagnetic scattering may result from diffraction, reflection, polarisation or refraction of the incident electromagnetic radiation including light and Xrays. In this regard, the beads may be formed of different materials to provide a set of beads with varying scattering properties such as different refractive indexes as for example described supra. Any suitable art recognised method of detecting and/or quantifying electromagnetic scatter may be employed. In this regard, the invention also contemplates methods employing contrast variation in light scattering as, for example, described in van Helden and Vrij (1980, Journal of Colloidal and Interface Science 76: 419-433), which is incorporated herein by reference.

Markers other than electromagnetic radiation-related markers can be utilised, optionally in combination with electromagnetic radiation-related markers. Such markers include e.g. size and/or shape of the bead. For example, beads may be shaped in the form of spheres, cubes, rectangular prisms, pyramids, cones, ovoids, sheets or cylinders, including intermediate forms as well as irregular shapes. Electrical impedance across a bead may be measured to provide an estimate of the bead volume (Coulter).

The marker in one embodiment comprises a chromophoric label. Suitable beads comprising such chromophores are described e.g. by Tentorio et al. (1980, Journal of Colloidal and Interface Science 77: 419-426), which is incorporated herein by reference.

A suitable method for non-destructive analysis of organic pigments and dyes, using a Raman microprobe, microfluorometer or absorption microspectrophotometer, is decribed for example in Guineau, B. (1989, Cent. Rech. Conserv. Documents

Graph., CNRS, Paris, Fr. Stud. Conserv 34 (1): 38-44), which is incorporated herein by reference.

Alternatively, the marker may comprise a magnetic material inclusive of iron and magnetite, or an marker that is detectable by acoustic backscatter as is known in the art.

It will be understood from the foregoing that the number of beads having different detectable codes will be dependent on the number of different detectable and/or quantifiable markers integrally associated with the beads.

Polymers

Polymers according to the present invention are preferably optically transparent in the optical exitation range of the fluorescent marker and/or the emission wavelength range of the fluorescent marker comprised by the particles and/or vacuoles of the polymer matrix.

The polymer is preferably selected from the group consisting of polyethers, polyvinyls, polyacrylates, polymethacrylates, polyacylamides, polyurethanes, polyacrylamides, polystyrenes, polycarbonates, polyesters, polyamides, and combinations threreof

More preferably, the polymer is selected from the group consisting of SPOCC, PEGA, HYDRA, POEPOP, PEG-polyacrylate copolymers, polyether-polyamine copolymers, crosslinked polyethylene diamines, and combinations thereof.

The beaded polymer matrix according to the invention preferably has a ratio R = a/b between a) the volume of the beaded or granulated polymer matrix and b) the average volume of the particles which is in the range of from 10000000:1 to 10:1, such as in the range of from 1000000:1 to 30:1, for example in the range of from 1000000:1 to 100:1, for example in the range of from 1000000:1 to 200:1, such as in the range of from 1000000:1 to 1000:1, for example in the range of from 100000:1 to 1000:1, such as in the range of from 100000:1 to 2000:1.

27-09-02

Independently of the above ratios, the beaded or granulated polymer matrix according to the invention preferably comprises an average volume of the swelled bead or granule of from 0.000001 µL – 50 µL, such as an average volume of the swelled bead or granule of from 0.00001 µL – 5 µL, for example an average volume of the swelled bead or granule of from 0.001 µL – 1 µL, such as an average volume of the swelled bead or granule of from 0.01 µL – 0.1 µL.

Any combination of the above falls within the invention and accordingly, for a ratio R = a/b between a) the volume of the beaded or granulated polymer matrix and b) the average volume of the particles which is in the range of from 10000000:1 to 10:1, such as in the range of from 1000000:1 to 30:1, for example in the range of from 1000000:1 to 100:1, for example in the range of from 1000000:1 to 200:1, such as in the range of from 1000000:1 to 1000:1, for example in the range of from 100000:1 to 1000:1, such as in the range of from 100000:1 to 2000:1, the average volume of the swelled bead or granule can be from 0.000001 µL – 50 µL, such as an average volume of the swelled bead or granule of from 0.00001 µL – 5 µL, for example an average volume of the swelled bead or granule of from 0.001 µL – 1 µL, such as an average volume of the swelled bead or granule of from 0.01 µL – 0.1 µL.

The invention is in one embodiment directed a plurality of beads comprising a population that is pre-encoded. Accordingly, each bead of that population has a code, which distinctively identifies a respective bead before, during and after said synthesis from other beads. The diversity of the said population of beads, therefore, resides in beads of said population having relative to each other different spatial locations of detectable markers, which are used to provide distinctive codes for each of those beads.

The beads of the invention may be used in many applications, such as combinatorial chemistry procedures that do or do not involve a split-and-combine procedure. Preferably, however, such assemblies are used in combinatorial chemistries, which involve a split-process-recombine procedure.

The beads may comprise any solid material capable of providing a base for combinatorial synthesis. For example, the beads may be polymeric supports such as polymeric beads, which are preferably formed from polystyrene cross-linked with 1-

5% divinylbenzene. Polymeric beads may also be formed from hexamethylenediaminepolyacryl resins and related polymers, poly N- {2- (4-hydroxylphenyl) ethyl} acrylamide (I. e., (one Q)), silica, cellulose beads, polystyrene beads poly (halomethylstyrene) beads, poly (halostyrene) beads, poly (acetoxystyrene) beads, latex beads, grafted copolymer beads such as polyethylene glycol/polystyrene, porous silicates for example controlled pore-glass beads, polyacrylamide beads for example poly (acryloylsarcosine methyl ester) beads, dimethylacrylamide beads optionally cross-linked with N, N'-bis-acryloyl ethylene diamine, glass particles coated with a hydrophobic polymer inclusive of cross-linked polystyrene or a fluorinated ethylene polymer which provides a material having a rigid or semi-rigid surface, poly (N-acryloylpyrrolidine) resins, Wang resins, Pam resins, Merrifield resins, PAP and SPARE polyamide resins, polyethylene functionalised with acrylic acid, kieselguhr/polyamide (Pepsyn K), polyHipeTM, PS/polydimethylacrylamide copolymers, CPG, PS macrobeads and TentageITM, PEG-PS/DVB copolymers.

The beads may be formed from appropriate materials inclusive of e.g. latex, glass and ceramic materials. Reference may also be made to W095/25737 and W097/15390, incorporated herein by reference, which disclose examples of such beads.

A plurality of beads according to the invention may be prepared by any suitable method. Preferably, when colloidal particles including polymeric and ceramic particles are used as beads, the colloid dispersion of such beads is stabilised. Exemplary methods imparting colloidal stabilisation are described for example in Hunter, R. J. (1986, "Foundation of Colloid Science", Oxford University Press, Melbourne) and Napper, D. H. (1983,"Polymeric stabilisation of Colloidal Dispersions"Academic Press, London), the disclosures of which are incorporated herein by reference. In this regard, the most widely exploited effect of nonionic polymers on colloid stability is steric stabilisation, in which stability is imparted by polymer molecules that are absorbed onto, or attached to, the surface of the colloid particles. Persons of skill in the art will recognise that it is possible to impart stability by combinations of different stabilisation mechanisms: e. g., surface charge on the particles can impact colloidal stability via electrostatic stabilisation, and an attached polyelectrolyte can impart stability by a combination of electrostatic and steric mechanisms (electrosteric stabilisation). Polymer that is in free solution can also influence colloid stability. Stabilisa-

tion by free polymer is well-documented (Napper 1983, supra) and is called depletion stabilisation.

Preferably, steric stabilisation of colloid dispersions is employed. In this regard, steric stabilisation is widely exploited because it offers several distinct advantages over electrostatic stabilisation. For example, one advantage is that aqueous sterically stabilised dispersions are comparatively insensitive to the presence of electrolytes because the dimensions of non-ionic chains vary relatively little with the electrolyte concentration.

Any suitable stabilising moiety may be used for stabilising colloidal dispersions. Exemplary stabilising moieties that impact on colloidal stability are given herein below: Poly (oxyethylene), Poly (vinyl alcohol), Poly (acrylic acid), Poly (acrylamide), and sorbitol monolaurate as well as commonly used emulsion stabilizers.

Encoded, beaded polymer matrix

It is another object of the invention to provide an encoded, beaded polymer matrix comprising individually detectable, spatially encoded beads, i.e. a composition of beads wherein essentially each bead of the encoded, beaded polymer matrix is uniquely identifiable.

The encoded, beaded polymer matrix preferably comprises at least 10^2 uniquely identifiable beads, such as at least 10^3 uniquely identifiable beads, for example at least 10^5 uniquely identifiable beads, such as at least 10^7 uniquely identifiable beads, for example at least 10^9 uniquely identifiable beads, such as at least 10^{11} uniquely identifiable beads, for example at least 10^{13} uniquely identifiable beads, such as at least 10^{15} uniquely identifiable beads, for example at least 10^{17} uniquely identifiable beads, such as at least 10^{19} uniquely identifiable beads, for example at least 10^{21} uniquely identifiable beads, such as at least 10^{23} uniquely identifiable beads.

The invention is also directed to a method for generating an encoded, beaded polymer matrix comprising the steps of:

(a) preparing a plurality of beads comprising spatially immobilised particles comprising at least one marker;
(b) detecting and/or quantifying the said markers of each bead and assigning a code, such as the result of a determination of the location of spatially encoded particles or vacuoles, for each bead;
(c) identifying beadss having distinctive codes; and optionally
(d) identifying beads having similar codes; and further optionally
(e) sorting the beads having distinctive codes from the beads having non distinctive codes to thereby provide an encoded, beaded polymer matrix.

There is also provided the use of such an encoded, beaded polymer matrix for identifying bioactive compound binding partners, and a use of the composition for diagnostic purposes, wherein the binding and determination of a predetermined binding partner to a substrate or bioactive compound on the carrier is at least indicative of a positive diagnosis.

Bioactive compounds of particular interest are e.g. those which may be so screened include agonists and antagonists for cell membrane receptors, toxins, venoms, viral epitopes, hormones, sugars, co-factors, peptides, enzyme substrates, drugs inclusive of opiates and steroids, proteins including antibodies, monoclonal antibodies, antisera reactive with specific antigenic determinants, nucleic acids, lectins, polysaccharides, cellular membranes and organelles.

The present invention also encompasses as bioactive compounds a plurality of unique polynucleotide or oligonucleotide sequences for sequence by hybridisation (SBH) or gene expression analyses. Persons of skill in the art will recognise that SBH uses a set of short oligonucleotide probes of defined sequence to search for complementary sequences on a longer target strand of DNA. The hybridisation pattern is used to reconstruct the target DNA sequence. Accordingly, in the context of the present invention, an aqueous solution of fluorescently labelled single stranded DNA (ssDNA) of unknown sequence may be passed over the library of polynucleotide or oligonucleotide compounds and adsorption (hybridisation) of the ssDNA will occur only on beads which contain polynucleotide or oligonucleotide sequences complementary to those on the ssDNA. These beads may be identified, for example, by flow cytometry, fluorescence optical microscopy or any other suitable technique.

27-09-02

Once a compound having the desired activity is obtained, the sequence of reaction steps experienced by the bead on which the compound was synthesised may be deconvoluted simply by analysing the tracking data for that bead as described, for example, hereinafter. The sequence of building blocks defining the compound of interest may thus be ascertained and a molecule comprising this sequence can by synthesised by conventional means (e. g., amino acid synthesis or oligonucleotide synthesis) as is known in the art.

Methods for generating an encoded, beaded polymer matrix

It is a further object of the invention to provide a method for generating an encoded, beaded polymer matrix, said method comprising the steps of

i) synthesizing a monomer and/or macromonomer and a crosslinker for polymerization, and,

ii) mixing the monomer and/or macromonomer with particles to give an even dispersion of particles in the mixture, and

iii) polymerizing the polymer by either i) suspension polymerisation and/or; ii) inverse suspension polymerisation and/or iii) bulk polymerisation followed by granulation and/or iv) droplet polymerisation.

The polymerisation reaction can preferably be a radical initiated chain polymerisation reaction, or an anion initiated ring opening polymerisation reaction, or a cation initiated ring opening polymerisation reaction.

Functional groups on beads of the encoded, beaded polymer matrix can subsequently be reacted with different bioactive compound building blocks as described herein elsewhere. Each reaction step can be monitored as essentially each bead of the encoded, beaded polymer matrix is individually detectable. The below methods describe in more detail the identification of spatially immobilised particles or beads in the beads or granules.

Polymer beads according to the invention can be prepared from a variety of polymerisable monomers, including styrenes, acrylates and unsaturated chlorides, esters, acetates, amides and alcohols, including, but not limited to, polystyrene (including high density polystyrene latexes such as brominated polystyrene), polymethylmethacrylate and other polyacrylic acids, polyacrylonitrile, polyacrylamide, polyacrolein, polydimethylsiloxane, polybutadiene, polyisoprene, polyurethane, polyvinylacetate, polyvinylchloride, polyvinylpyridine, polyvinylbenzylchloride, polyvinyltoluene, polyvinylidenechloride and polydivinylbenzene, as well as PEGA, SPOCC and POEPOP. The beads may be prepared from styrene monomers or PEG based marcromonomers.

Ceramic beads may be comprised of silica, alumina, titania or any other suitable transparent material. Preferably, silica particles are employed. A suitable method of making silica beads is described, for example in"The Colloid Chemistry of Silica and Silicates" (Cornell University Press) by Ralph K Iler 1955 and U. S. Patent No 5,439,624, the disclosures of which are incorporated herein by reference.

Fluorescent dyes may be incorporated into beads by any suitable method known in the art, such as copolymerisation of a polymerisable monomer and a dyecontaining co-monomer or addition of a suitable dye derivative in a suitable organic solvent to an aqueous suspension as for example disclosed in Singer et al., (supra including references cited therein), Camplan et al. (1994, In"Innovation and Perspectives on Solid Phase Synthesis"Epton, R., Birmingham: Mayflower, 469-472, incorporated herein by reference) and Egner et al. (1997, Chem. Commun. 73 5-73 6, incorporated herein by reference). Alternatively, fluorescent beads may be produced having at least one fluorescent spherical zone. Such particles may be prepared as for example described in U. S. Patent No. 5,786,219 (Zhang et al.), which is incorporated herein by reference. In a preferred embodiment, one or more fluorescent dyes are incorporated within a microparticle. Compared to surface attachment of fluorescent dyes, incorporation of dyes within beads reduces the physical exposure of the fluorescent dye (s) to various solvents used in combinatorial synthesis and thus increases the stability of the beadfluorescent dye complexes.

27-09-02

Methods for identifying spatially immobilised particles or vacuoles

In one embodiment, the spatial immobilisation of the plurality of particles in each beaded polymer matrix is essentially unique for each bead. The spatial positions of particles in each bead can be defined by sets of coordinates, (x,y,z) of particle centers of said particles, relative to one reference point of the detection. Furthermore, the relative positions in space of centers (x,y,z) of immobilized particles can be detected based on recording of 2D-projections of the particles. 3 2D-projections can be recorded along 3 orthogonal axis x, y and z to generate 3 sets of 2D-coordinates (y,z), (x,z) and (x,y), respectively, from which the 3D-coordinates (x,y,z) of particle centers can be derived. A stack of 2D projections can be generated by confocal or focal microscopy to recreate the 3D image matrix of the bead from which the relative particle position (x,y,z) in space can be determined.

One method for determination of relative particle positions within a bead can be based on focussed scanning lasers and laminar fluidics, preferably methods in which the coordinates x and y of a particle position is determined by fast scanning two orthogonally aligned lasers over two cross sections of the moving bead while the z coordinate is determined by the time of flight of the bead at known flow rates. Accordingly, it is possible to determine the coordinates x and y of a particle position by using a single laser and a rotating mirror that via 2 or three geometrically arranged static mirrors reflects the laser beam along 2 or 3 orthogonal axis

In yet another embodiment, the present invention provides a method for identifying at least one uniquely identifiable, spatially encoded bead of a polymer matrix, said method comprising the steps of

i) determining the unique, spatial immobilisation of a plurality of particles in the at least one bead to be identified, and

ii) identifying said at least one uniquely identifiable, spatially encoded beaded polymer matrix based on said unique determination of said spatially immobilised plurality of particles.

27-09-02

A binding assay for characterising or isolating active compounds bound to the beads or granules can be performed by measuring e.g. the binding of a protein to a ligand bound to the polymer matrix. Also, an assay can be performed by measuring e.g. an enzyme activity on a substrate bound to the polymer matrix. It is also possible to perform an assay by measuring e.g. enzyme inhibition of a molecule bound to the polymer matrix, or to perform an assay by measuring e.g. receptor interaction with a bioactive compound bound to the polymer matrix.

For the above methods, the plurality of particles preferably comprise a fluorescently detectable marker, such as a fluorescently detectable marker detectable by two photon fluorescence microscopy, or a fluorescently detectable marker detectable by one photon fluorescence microscopy.

Method for generating an encoded, beaded polymer matrix comprising different bioactive compounds

It is a yet further object of the invention to provide a method for generating an encoded, beaded polymer matrix comprising a bioactive compound, wherein essentially each bead of the polymer matrix is uniquely identifiable, said method comprising the steps of

i) spatially immobilizing particles in polymer beads or granulates, and

ii) isolating labelled beads or granules by automated sorting, and

iii) recording and storing the distance matrix for essentially each bead or granule, and

iv) performing a stepwise synthesis of bioactive compounds by reacting functional groups of the encoded beads or granules with at least one building block, and

v) recording the identity of each bead or granule that enter each reaction step iv), and

vi) Isolating beads or granules of interest, preferably by performing an assay or a diagnostic screen, and

vii) Identifying the bioactive compound attached to at least one individual bead by recording the identity of at least one isolated bead or granule, and optionally comparing said recording with the recording, preferably a distance matrix, recorded for at least a plurality of the remaining beads or granules.

In a further aspect, the invention provides a method for synthesising and deconvoluting a combinatorial library comprising the steps of :

(a) apportioning in a stochastic manner among a plurality of reaction vessels a plurality of beads on which a plurality of different compounds can be synthesised, wherein said plurality of beads comprises a population of detectably distinct beads each having a code, such as spatially immobilised particles or vacuoles, which distinctively identifies a respective bead before, during and after said synthesis from other beads,
(b) determining and recording the codes, preferably in the form of the spatial position of the immobilised particles or vacuoles, of said plurality of beads in order to track the movement of individual detectably distinct beads into particular reaction vessels of said plurality of reaction vessels, wherein said codes are determined prior to step (d);
(c) reacting the beads in each reaction vessel with a building block;
(d) pooling the beads from each reaction vessel;
(e) apportioning the beads in a stochastic manner among the plurality of reaction vessels;
(f) reacting the beads in each reaction vessel with another building block;
(g) recording the codes of said plurality of beads in order to track the movement of individual detectably distinct beads into particular reaction vessels of said plurality of reaction vessels, wherein said codes are recorded after step (e) and/or step (f);
(h) pooling the beads from each reaction vessel;
(i) iterating steps (e) through (h) as required in order to create a combinatorial compound library wherein member compounds of the library are associated with the detectably distinct beads and wherein codes of the detectably distinct beads are

deconvolutable using tracking data provided by said recordal steps to identify the sequence of reactions experienced by the said detectably distinct beads.

Bead analysis

The identification steps (step (c) and (d)) may be effected by use of any suitable method or apparatus for analysing the spatially immobilised markers of a bead.

Preferably, these steps are effected by flow cytometry, which typically detects optical parameters. For example, a flow cytometer may be used to determine forward scatter (which is a measure of size of a bead), side scatter (which is sensitive to refractive index and size of a particle (seen Shapiro 1995,"Practicalflow cytometry", 3d ed. Brisbane, Wiley-Liss)), and fluorescent emission.

Any suitable algorithm may be employed to track and/or sort individual detectably unique beads. Preferably, a real-time algorithm is employed.

Suitably, the step of sorting (step (e)) is characterised in that the population of detectably distinct beads constitutes at least about 50%, preferably at least about 70%, more preferably at least about 90%, and more preferably at least about 95% of the plurality of beads resulting from step (e).

From the foregoing, a population of detectably unique beads can be generated from a raw population of beads using e.g. specialised flow cytometric techniques. The population of detectably unique beads is thereby "pre-encoded" and can be used for combinatorial synthesis.

Building block reactions

The beads of the invention are applicable to any type of chemical reaction that can be carried out on a solid support. Such chemical reaction includes, for example:

1. 2 + 2 cycloadditions including trapping of butadiene;
2. [2 + 3] cycloadditions including synthesis of isoxazolines, furans and modified peptides;
3. acetal formation including immobilization of diols, aldehydes and ketones;

4. aldol condensation including derivatization of aldehydes, synthesis of propanediols;
5. benzoin condensation including derivatization of aldehydes;
6. cyclocondensations including benzodiazepines and hydantoins, thiazolidines, -turn mimetics, porphyrins, phthalocyanines;
7. Dieckmann cyclization including cyclization of diesters;
8. Diels-Alder reaction including derivitisation of acrylic acid ;
9. Electrophilic addition including addition of alcohols to alkenes;
10. Grignard reaction including derivatisation of aldehydes;
11. Heck reaction including synthesis of disubstituted alkenes;
12. Henry reaction including synthesis of nitrile oxides in situ (see 2 + 3 cycloaddition);
13. catalytic hydrogenation including synthesis of pheromones and peptides (hydrogenation of alkenes);
14. Michael reaction including synthesis of sulfanyl ketones, bicyclo] 2.2.2] octanes;
15. Mitsunobu reaction including synthesis of aryl ethers, peptidyl phosphonates andthioethers;
16. nucleophilic aromatic substitutions including synthesis of quinolones;
17. oxidation including synthesis of aldehydes and ketones;
18. Pausen-Khand cycloaddition including cyclization of norbornadiene with pentynol;
19. photochemical cyclisation including synthesis of helicenes;
20. reactions with organo-metallic compounds including derivitisation of aldehydes and acyl chlorides;
21. reduction with complex hydrides and Sn compounds including reduction of carbonyl, carboxylic acids, esters and nitro groups;
22. Soai reaction including reduction of carboxyl groups;
23. Stille reactions including synthesis of biphenyl derivatives;
24. Stork reaction including synthesis of substituted cyclohexanones;
25. reductive amination including synthesis of quinolones;
26. Suzuki reaction including synthesis of phenylacetic acid derivatives; and
27. Wittig, Wittig-Horner reaction including reactions of aldehydes; pheromones and sulfanyl ketones.

27-09-02

Reference may also be made to Patel et al., (April 1996; DDT 1 (4): 134-144) who describe the manufacture or synthesis of N-substituted glycines, polycarbamates, mercaptoacylprolines, diketopiperazines, HIV protease inhibitors, 1-3 diols, hydroxystilbenes, B-lactams, 1,4-benzodiazepine-2-5-diones, dihydropyridines and dihydropyrimidines.

Reference may also be made to synthesis of polyketides as discussed, for example, in Rohr (1995, Angew. Int. Ed. Engl. 34: 881-884).

Chemical or enzymatic synthesis of the compound libraries of the present invention takes place on beads. Thus, those of skill in the art will appreciate that the materials used to construct the beads are limited primarily by their capacity for derivitisation to attach any of a number of chemically reactive groups and compatibility with the chemistry of compound synthesis. Except as otherwise noted, the chemically reactive groups with which such beads may be derivatised are those commonly used for solid state synthesis of the respective compound and thus will be well known to those skilled in the art. For example, these bead materials may be derivatised to contain functionalities or linkers including-NH2, -NHNH2, -ONH2, -COOH, -SH, -SeH, -SO3H, -GeH, or -SiR2H groups.

Linkers for use with the beads may be selected from base stable anchor groups as described in Table 2 of Fruchtel et al. (1996, supra, the entire disclosure of which is incorporated herein by reference) or acid stable anchor groups as described in Table 3 of Fruchtel et al. (1996, supra). Suitable linkers are also described in WO93/06121, which is incorporated herein by reference.

In the area of peptide synthesis, anchors developed for peptide chemistry are stable to either bases or weak acids, but for the most part, they are suitable only for the immobilisation of carboxylic acids. However, for the reversible attachment of special functional groups, known anchors have to be derivatised and optimised or, when necessary, completely new anchors must be developed. For example, an anchor group for immobilisation of alcohols is (6 hydroxymethyl)-3,4 dihydro-2H-pyran, whereby the sodium salt is covalently bonded to chloromethylated Merrifieldz resin by a nucleophilic substitution reaction. The alcohol is coupled to the support by electrophilic addition in the presence of pyridinium toluene-4 sulphonate (PPTS) in

dichloromethane. The resulting tetrahydropyranyl ether is stable to base but can be cleaved by transetherification with 95% trifluoroacetic acid. Benzyl halides may be coupled to a photolabile sulfanyl-substituted phenyl ketone anchor.

It will also be appreciated that compounds prepared with the beads and/or process of the present invention may be screened for an activity of interest by methods well known in the art. For example, such screening can be effected by specialised flow cytometry invented from standard techniques such as described e.g. by Needels et al. (1993, Proc. Natl. Acad. Sci. USA 90: 1070010704, incorporated herein by reference), Dower et al. (supra), and Kaye and Tracey (WO 97/15390, incorporated herein by reference).

Synthesis of a combinatorial compound library

The invention also resides in a method of synthesising and deconvoluting a combinatorial library. The codes of the plurality of beads are determined preferably before the first reaction step, although codes may be determined at any time before the first pooling step (step (d), cf. method steps cited above).

Preferably, every time the plurality of beads is apportioned into reaction vessels, each one of the vessels is analysed to determine which of the detectably distinct beads are in each reaction vessel. A database of all the beads (or corresponding gridspaces, supra) can thus be updated to show the synthetic history of the compound synthesised on each bead.

During a reaction step, the beads in each reaction vessel are reacted with a building block required to assemble a particular compound. Assembly of compounds from many types of building blocks requires use of the appropriate coupling chemistry for a given set of building blocks. Any set of building blocks that can be attached to one another in a step-by-step fashion can serve as the building block set. The attachment may be mediated by chemical, enzymatic, or other means, or by a combination of these. The resulting compounds can be linear, cyclic, branched, or assume various other conformations as will be apparent to those skilled in the art. For example, techniques for solid state synthesis of polypeptides are described, for example, in Merrifield (1963, J. Amer. Chem. Soc. 35: 2149-2156). Peptide coupling chemistry is

also described in"The Peptides", Vol. 1, (eds. Gross, E., and J. Meienhofer), Academic Press, Orlando (1979), which is incorporated herein by reference.

To synthesise the compounds, a large number of the beads are apportioned among a number of reaction vessels. In each reaction, a different building block is coupled to the growing oligomer chain. The building blocks may be of any type that can be appropriately activated for chemical coupling, or any type that will be accepted for enzymatic coupling.

Because the reactions may be contained in separate reaction vessels, even building blocks with different coupling chemistries can be used to assemble the oligomeric compounds (see, The Peptides, op. cit). The coupling time for some of the building block sets may be long. For this reason the preferred arrangement is one in which the building block reactions are carried out in parallel. After each coupling step, the beads on which are synthesised the oligomers or compounds of the library are pooled and mixed prior to re-allocation to the individual vessels for the next coupling step. This shuffling process produces beads with many oligomer sequence combinations. If each synthesis step has high coupling efficiency, substantially all the oligomers on a single bead will have the same sequence. That sequence is determined by the synthesis pathway (building blockreactions and the order of reactions experienced by the beads) for any given bead. The maximum length of the oligomers may be about 50, preferably from 3 to 8 building blocks in length, and in some cases a length of 10 to 20 residues is preferred. Protective groups known to those skilled in the art may be used to prevent spurious coupling (see, The Peptides, Vol. 3, (eds. Gross, E., and J. Meienhofer), Academic Press, Orlando (1981), which is incorporated herein by reference).

With enough beads and efficient coupling it is possible to generate complete sets of certain oligomers, if desired. The appropriate size of the beads depends on (1) the number of oligomer synthesis sites desired; (2) the number of different compounds to be synthesised (and the number of beads bearing each oligomer that are needed for screening); (3) the effect of the size of the beads on the specific screening strategies e. g. fluorescence-activated cell sorters (FACS) to be used; and (4) the resolution of the encoding/detection methods employed.

27-09-02

Kit of parts

The invention in a still further aspect resides in a kit comprising:

(a) a combinatorial compound library including a plurality of different compounds wherein each compound is attached to at least one of a plurality of beads,
which includes a population of detectably distinct beads each having a distinctive code, which distinctively identifies a respective bead before, during and after synthesis of a corresponding compound from other beads; and
(b) tracking data on each distinctive code to identify the sequence of reactions experienced by a respective detectably distinct bead.

Examples

General Methods.

Reagents were obtained from Fluka and used without any purification. All solvents used were of HPLC grade kept over molecular sieves. Oregon green was obtained from Molecular Probes. The 28-53 µm beads were prepared in a specially designed high-speed stirred polymerisation steel reactor and 5-28 µm beads were prepared by using a high-speed dispersion reactor. The encoded macro beads were prepared in a 250 ml baffled glass reactor equipped with a dispersion stirrer. The fluorescence images were obtained with a microscope and a digital camera. Broad band excitation in the near UV range was provided by a mercury lamp. The images of the encoded beads were recorded in water.

Example 1: Preparation of encoded $(NH_2)_2PEG_{1900}$-Acrylamide copolymer beads.

Labelled microbeads encoded $(Acr)_{1.4}$ $(NH_2)_2PEG_{1900}$-acrylamide were prepared by inverse suspension polymerisation method. In order to prepare the beads having a size 500 µm, a lower wt% (1.4%) of sorbitan monolaurate with the macromonomer was used as the suspension stabiliser. The n-heptane was used as the suspension

medium and was degassed with argon for 1 h before the addition of monomers. In a typical synthesis procedure, a solution of $(Acr)_{1.4}$ $(NH_2)_2PEG_{1900}$ (7.3 g, 3.54 mmol) in water (21 mL) was degassed with argon for 30 min. Acrylamide (0.36 g, 5 mmol) and the labelled micro beads (20 mg) in water (0.5 mL) were added to the degassed solution and the purging of argon was continued for 5 min. A solution of sorbitan monolaurate (0.1 mL) in DMF (1 mL) and the free radical initiator ammonium persulfate (300 mg) in water (2 mL) were added to the monomer mixture. The reaction mixture was then rapidly added to the suspension medium and stirred at 600 rpm at 70 °C. After one min, TEMED (1.5 mL) was added to the reactor. The reaction was allowed to continue for 3h, the encoded beads formed were filtered through the sieves and the 500 µm fraction was collected. The beads were washed thoroughly with ethanol (10x), water (10x), ethanol (10x) and dried under high vacuum.

Example 2: Preparation of microbeads for encoding

Synthesis of partially acryloylated $(NH_2)_2PEG_{500}$ and $(NH_2)_2PEG_{1900}$

Acryloyl chloride (1.267 mL, 14 mmol) in DCM (12 mL) was added dropwise to a solution of $(NH_2)_2PEG_{500}$ (6.3 g, 10 mmol) in DCM (18 mL) at 0 °C with stirring. The reaction mixture was kept for 1 h at 20 °C. The DCM was evaporated and drying in vacuo at 20 °C yielded the 70% acryloylated $(NH_2)_2PEG_{500}$ as colourless thick oil. The partially acryloylated $(NH_2)_2PEG_{1900}$ was prepared by following the same procedure with $(NH_2)_2PEG_{1900}$ (20 g, 10 mmol) in DCM (12 mL) and acryloyl chloride (1.267 mL, 14 mmol) in DCM (18 mL).

Synthesis of $(Acr)_{1.4}$ $(NH_2)_2PEG_{500}$-DMA micro beads (28-53 µm) :

A: using high speed stirred reactor:

Beads of $(Acr)_{1.4}$ $(NH_2)_2PEG_{500}$-DMA (28-53 µm) were prepared by the inverse suspension polymerisation of aqueous solutions of monomers in n-heptane:CCl_4 mixture(6:4, v/v, 240 mL). Sorbitan monolaurate was used by 8 wt % of the macromonomer for the stabilisation of the suspension. Argon was bubbled to the n-hepane-CCl_4 mixture for 1 h before the addition of monomers. In a typical synthesis procedure, a solution of $(Acr)_{1.4}$ $(NH_2)_2PEG_{500}$ (6.6 g, 10 mmol) in water (21 mL) was degassed with argon for 30 min. DMA (343µL, 3.32 mmol) was added to the degassed solution and the purging of argon was continued for 5 min. A solution of sor-

bitan monolaurate (0.5 mL) in DMF (2 mL) and the free radical initiator ammonium persulfate (200 mg) in distilled water (1 mL) were added to the monomer mixture. The reaction mixture was then rapidly added to the suspension medium in the polymerisation reactor stirred at 2500 rpm at 70 °C. After one min, TEMED (1 mL) was added to the reactor. The reaction was allowed to continue for 3h, the microbeads formed were filtered through the sieves and the 28-53 µm fractions were collected. The microbeads were washed thoroughly with ethanol (10x), water (10x), ethanol (10x) and dried under high vacuum.

B: using dispersing instrument

Microbeads of $(Acr)_{1.4}$ $(NH_2)_2PEG_{500}$ (5-28 µm) were prepared by the inverse suspension polymerisation of aqueous solutions of monomer in n-heptane (100 mL). Sorbitan monolaurate was used by 25 wt % of the macromonomer for the stabilisation of the suspension. Argon was bubbled to the n-hepane for 1 h before the addition of monomer. A solution of $(Acr)_{1.4}$ $(NH_2)_2PEG_{500}$ (2 g, 3.03 mmol) in water (6 mL) was degassed with argon for 30 min. A solution of sorbitan monolaurate (0.5 mL) in DMF (1 mL) and the free radical initiator ammonium persulfate (200 mg) in distilled water (1 mL) were added to the monomer solution. The reaction mixture was then rapidly added to the suspension medium in a reactor equipped with a high-speed dispersing instrument stirred at 9000 rpm at 70 °C. After one min, TEMED (1 mL) was added to the reactor. The reaction was allowed to continue for 3h, the microbeads formed were filtered through the sieves and the 5-28 µm fractions were collected. The microbeads were washed thoroughly with ethanol (10x), water (10x), ethanol (10x) and dried under high vacuum.

Example 3: Labelling of encoding particles:

Labelling of $(Acr)_{1.4}$ $(NH_2)_2PEG_{500}$-DMA (28-53 µm) micro beads with Oregon Green 514 dye:

The microbeads (0.2 g, 0.8 mmol/g) were kept in DMF/water (5 mL) for 1h. The Oregon Green™514 carboxylic acid, succinimidyl ester (0.147 g, 0.24 mmol) in DMF (200 µL) was added to the swollen microbeads and the reaction mixture was kept at room temperature. After 1 h, the beads were filtered through a 0.45 micron filter and washed with DMF (10x) and water (10x).

27-09-02

Labelling of $(Acr)_{1.4}$ $(NH_2)_2PEG_{500}$ (5-28 µm) micro beads with Oregon Green 514 dye

The microbeads (0.2 g, 1 mmol/g) were kept in DMF/water (5 mL) for 1h. The Oregon Green™514 carboxylic acid, succinimidyl ester (0.184 g, 0.3 mmol) in DMF (200 µL) was added to the swollen microbeads and the reaction mixture was kept at room temperature. After 1 h, the beads were filtered through a 0.45 micron filter and washed with DMF (10x) and water (10x).

Labelling of $(Acr)_{1.4}$ $(NH_2)_2PEG_{500}$-DMA (28-53 µm) micro beads with 1-Cyano benz[f]isoindole

To a stirred suspension of 2,3-naphthalene dicarboxaldehyde (29.44 mg, 0.16 mmol) in MeOH (2 mL) was added NaCN (8 mg, 0.16 mmol) at room temperature. To the reaction mixture, the resin (0.2 g, 0.16 mmol) was added and kept at room temperature. After 30 min, the resin was filtered and washed with MeOH (10x), DMF (10x) and water (10x).

Example 4: Presorting of encoded beads according to integrated fluorescence.

A custom made Compas Beads (Union Biometrica) beadsorter with laser exitation at 488 nm and detection of fluorescence at 514 nm was used to pool the beads synthesized according to the number if small particles. The integrated fluorescence of the small particles was recorded with the selection (sorting) window preset to collect those beads having 3-4 small particles at a sorting rate of 30 beads / s at a flowrate of 1000 mm / s of Compas sheat fluid corresponding to sorting of 900.000 beads in a working day. The collected beads were resorted to yield approximately ~20% containing 3 – 4 particles / bead. The quality of the collected pool was veryfied usinga fluorescence microscope.

Example 5: Synthesis of 400 dipeptides with bead portioning and identification.

The peptide library was prepared in a 20-well multiple column peptide synthesiser. Approximately 50 beads were taken in a glass plate and the image of these beads were recorded in three shuffled states and then added to one of the wells in the

synthesiser. The beads were taken in 20 wells of the synthesiser accordingly. The resin was washed with DMF and the N_α-Fmoc-protected OPfp ester of the amino acid (10 mg) was added to each well of the synthesis block. The reaction mixture was kept at room temperature for 3 h and washed with DMF (6x). The beads were removed from the block, combined together and the Fmoc-protection was removed by 20% piperidine in DMF (3mL, 20 min). The resin was washed with DMF (10x), split in to 20 portions and added to each well of the block after recording its image in three shuffled states. After the incorporation of the second amino acid, the beads were transferred to a syringe. The Fmoc protection was removed by 20% piperidine in DMF (3 mL, 20 min) and the resin was washed with DMF (10x). The side chain protection of the peptide was removed by treating with 50% TFA in DCM (3 mL, 30 min), and the resin was washed with DCM (10x), DMF (10x) and water (10x).

Example 6: Selection and structure determination on a fraction of an encoded library by visual decoding.

Twenty beads were randomly selected from the peptidyl resin and record the images separately in water. The sequence of the dipeptide on each bead was decoded by visual comparison of final image of the bead with pre-recorded images of the beads.

Example 7: Confirmation of structure by solid phase Edman sequencing.

Single beads from the dipeptide library were placed on a filter and subjected to Edman sequencing on a 477 A Protein Sequencer (Applied Biosystems) to provide the dipeptide structure in two standard cycles.

Example 8: Capturing 3 orthogonal 2D-projections of a bead

A triangular hole was carved in a 1 cm plate of POM. The hole was symmetrical with sides angled at 54.3 ° and a length of the side of the lower triangle of 5 mm. The plate was mounted horisontally and a microscope was mounted at an angle of 35.7 ° underneath so it was perpendicular to the surface of a quartz flowcell mounted in the triangular hole of the POM holder. The corner of the quartz cell could thus be projected onto the CCD of the microscope from all three orthogonal sides, simple by carefull rotation of the quartz cell. The beads recorded were fixed on the quartz cell

wall simply by adhesion to the walls of the corner and was submerged in the appropriate solvent. Three orthogonal pictures were recorded under identical conditions and the coordinates relative to one of the particles were generated.

Example 9: Determining the centre of a particle in a 2-D projection.

The three 2-D pixel-based projections obtained from CCD cameras are treated by the following algorithm. For each alternate pixel in each alternate line of the image it was tested whether a pixel was bright or dark by testing the blue rgb value. Testing was continued from the one before the bright pixel in single pixel steps until at least two dark pixels were detected. Then the center of the range of bright pixels were determined. From this point the height and the center of the bright spot was determined. The center and the region occupied by the bead was recorded. The search for spots was continued, but ommitting bright pixels within areas already occupied. The centers of bright spots with an area above a small treshold were used as coordinates for the particles.

Example 10: Confocal determination of spatial positions of particles in a bead

In a stopped flow system individual beads are positioned in a confocal scanning system as in a commercial scanning microscope. The positioning system is based on small IR lasers detecting changes in refractive index or absorption. If the particles are fluorescent, the bead is illuminated with monochromatic light corresponding to excitation wavelength of the fluorescent dye. If it is simple coloured particles the bead is illuminated with white light.

The bead is scanned in consecutive 2-D layers as depicted in figure 3. The resolution (pixel dimension) in the layers as well as the distance between layers is selected to be smaller than the average particle diameter. Based on the consecutive scans a 3-D matrix of particle positions are formed. The dataset is reduced calculating inter-particle distances or vectors, and the remaining information is discarded.

Example 11: Recording of coordinates of particles in a moving bead by two alternating scanning lasers

In a pulsation free constant flow system individual beads are passed through a scanning system with two orthogonal laser scanning systems as depicted in figure 4. The two 1-D laser scanners are both orthogonal to the flow direction, and allow a full 3-D scanning of the passing beads, which are moving with constant velocity. In fluorescence mode the lasers will emit light at an excitation wavelength for the fluorescent dye in the particles in the beads.

A fast response emission light detector records the time-resolved emission signal, which in conjunction with the flow speed and the scan parameters are used to construct a full 3-D matrix of particle positions. The dataset is reduced calculating inter-particle distances or vectors, and the remaining information is discarded.

Example 12: Recording of coordinates of particles in a moving bead by rotational scan

In a pulsation free constant flow system individual beads is passed a scanning system applying a rotational scan focussed on the bead via a parabolic mirror as depicted in figure 4.

The two 1-D laser scanners are both orthogonal to the flow direction, and allow a full 3-D scanning of the passing beads, which are moving with constant velocity. In fluorescence mode the laser emits light at an excitation wavelength for the fluorescent dye in the particles in the beads. The circle scan onto the parabolic mirror is passing alternating 60 degree segments of full and blocked transmission to give three curved scan lines for each rotation.

A fast response emission light detector records the time-resolved emission signal, which in conjunction with the flow speed and the scan parameters are used to construct a 3-D matrix of particle positions. The dataset is reduced calculating inter-particle distances or vectors, and the remaining information is discarded.

Example 13: Determination of distance matrix from particle coordinates.

The coordinates of the particles were determined according to example 8 above. Coordinates were measured in pixel units e.g for picture set b1a-b1c coordinates (0, 0, 0); (22, 110, 84); (-94, 168, 153) were measured. From these coordinates the unique set of distance parameters (140, 245, 146) (length of inter particle vectors) were calculated according to the formula presented in Figure 1. The average error on determination of coordinates was approximately 2 % corresponding to the resolution of the method.

Example 14: Robustness of the method for identification.

In order to obtain a quantitative measure of how well individual beads can be distinguished by inserting small fluorescent beads to encode them, a Monte Carlo simulation was performed.

To this end, envisage a spherical bead as being composed of small cubic volume elements (voxels) of unit vertex length. The actual voxel size corresponds to the accuracy of determining the position of the fluorescent marker within the bead. Then the voxel centers form a grid of potential encoding points within the spherical bead, out of which in the simplest cases m=3 or m=4 are randomly selected. Generically, these points of encoding can be regarded as corners of a triangle with n=3 vertices or of a tetrahedron (n=6 vertices). The vertex lengths correspond to the distances between the encoding points. When ordered by magnitude, the set of vertex lengths is invariant under global rotation and translation of the large bead. (Moreover, it is in fact invariant under any action of a symmetry point group in three-dimensional space including inversion at the origin and mirroring at a plane thus discarding potential discrimination by chirality in the case of tetrahedra.)

If the set of vertex lengths is ordered in descending magnitude, i.e. $v_1 \geq v_2 \geq \ldots \geq v_n \geq 0$ with $n = 3\,(6)$, then the encoding vector $\mathbf{v} = (v_1, v_2, \ldots, v_n)$ can be identified with a point in the upper half of the three- (or six-) dimensional positive real space $\mathbb{R}^3_+$ ($\mathbb{R}^6_+$), respectively.

Let $r \geq 0$ denote the finite resolution of a CCD camera. Then two beads j and k cannot be discerned if their encoding vectors $\mathbf{v}_j$ and $\mathbf{v}_k$ correspond to points less than r apart from one another, i.e. if the separation of encoding $\Delta v = \tau\, \mathbf{v}_j - \mathbf{v}_k \tau$ falls below the camera resolution $\Delta v \leq r$.

27-09-02

The corresponding Monte Carlo simulation gives the following results for a bead of 500 units diameter: If the bead is marked with 3 fluorescent points, then 5 in 10'000 beads give rise to an encoding separation ∩v P 6 units. If 4 encoding points are used as shown in Fig. 8, then only 2 in 10'000 beads show separations ∩v P 12 units, and in none out of 50 million pairs simulated the separation is ∩v P 3 units. For a bead diameter of 5000 units the resolution greatly improves: Encoding by 3 points leads to 5 beads in 100'000 whose separations of encoding are ∩v P 60 units. With 4 points one only has 1 in 100'000 beads with ∩v P 120 units separation.

In conclusion, by inserting four encoding particles into a standard bead of 500 µm diameter, within which the centers of the fluorescent particles can be determined with a typical accuracy of 1 µm, provides ample space for encoding many millions of individual beads. The probability that an active hit will not be uniquely identified is very small according to the present simulations.

Claims

1. An encoded beaded or granulated polymer matrix for solid phase synthesis comprising beads or granules each comprising a plurality of spatially immobilised particles or vacuoles, wherein each particle or vacuole is individually detectable.

2. The beaded or granulated polymer matrix according to claim 1 in which the matrix has different optical or spectroscopic properties from those of the immobilised particles or vacuoles.

3. The beaded or granulated polymer matrix according to claim 1 in which the immobilised particles or vacuoles are monodisperse

4. The beaded or granulated polymer matrix according to claim 1 in which the immobilised particles are heterodisperse

5. The beaded or granulated polymer matrix according to claim 1 in which the immobilised particles are regular spherical beads or vacuoles

6. The beaded or granulated polymer matrix according to claim 1 in which the immobilised particles are irregular particles.

7. The beaded or granulated polymer matrix according to claim 1, wherein each beaded or granulated polymer matrix comprises at least 2 particles.

8. The beaded or granulated polymer matrix according to claim 1, wherein each beaded or granulated polymer matrix comprises at least 3 particles.

9. The beaded or granulated polymer matrix according to claim 1, wherein each beaded or granulated polymer matrix comprises at least 4 particles.

10. The beaded or granulated polymer matrix according to any of claims 7 to 9, wherein said particles are essentially spherical.

27-09-02

11. The beaded or granulated polymer matrix according to any of claims 7 to 10, wherein at least two of said particles have essentially the same diameter.

12. The beaded or granulated polymer matrix according to any of claims 10 and 11, wherein all of said particles are essentially monodisperse.

13. The beaded or granulated polymer matrix according to any of claims 10 to 12, wherein said particles are less than 10 micrometer in diameter.

14. The beaded or granulated polymer matrix according to claim 13, wherein said particles are less than 5 micrometer in diameter.

15. The beaded or granulated polymer matrix according to claim 13, wherein said particles are less than 1 micrometer in diameter.

16. The beaded or granulated polymer matrix according to claim 13, wherein said particles are less than 0.1 micrometer in diameter.

17. The beaded or granulated polymer matrix according to any of claims 10 to 12, wherein said particles comprise a spectroscopically detectable marker.

18. The beaded or granulated polymer matrix according to any of claims 10 to 12, wherein said particles comprise a fluorescently detectable marker.

19. The beaded or granulated polymer matrix according to claim 18, wherein said fluorescently detectable marker is selected from the fluorescent group of compounds and materials consisting of fluorencent organic polycyclic compounds, conjugated vinylic compounds, heterocyclic transition metal complexes, rare earth metal compounds, inorganic oxides and glasses.

20. The beaded or granulated polymer matrix according to claim 18, wherein said fluorescently detectable marker is detectable by two photon fluorescence spectroscopy.

27-09-02

21. The beaded or granulated polymer matrix according to claim 18, wherein said fluorescently detectable marker is detectable by one photon fluorescence spectroscopy.

22. The beaded or granulated polymer matrix according to claim 18, wherein said fluorescently detectable marker is detectable by time-correlated photon fluorescence spectroscopy

23. The beaded or granulated polymer matrix according to claim 1, wherein the polymer is optically transparent in the optical exitation of said fluorescent marker and/or the emission wavelength ranges of said fluorescent marker.

24. The beaded or granulated polymer matrix according to claim 1, wherein the polymer is selected from the group consisting of polyethers, polyvinyls, polyacrylates polyacylamides polyacrylamides, polystyrenes, polycarbonates, polyesters, polyamides, and combinations threreof

25. The beaded polymer matrix according to claim 1, wherein the polymer is selected from the group consisting of SPOCC, PEGA, HYDRA, POEPOP, PEG-polyacrylate copolymers, polyether-polyamine copolymers, crosslinked polyethylene diamines, and combinations thereof.

26. The beaded polymer matrix according to claims 1, wherein said particles comprise a marker, which is detectable by fast spectroscopic techniques other than fluorescence spectroscopy.

27. The beaded polymer matrix according to claims 26, wherein said fast spectroscopic technique comprise infrared spectroscopy, raman spectroscopy, visble light spectroscopy, UV spectroscopy, electron spin resonance, and nuclear magnetic resonance.

28. The beaded polymer matrix according to claims 1, wherein said particles comprise a marker, which is detectable by fast detection techniques other than spectroscopy such as light scattering, reflection, diffraction or light rotation.

27-09-02

29. Method for spectroscopically detecting a marker comprised in the matrix according to any of claims 1 to 28, said method comprising the step of probing the marker by a range of frequencies differing by less than 10% based on the numerical highest frequency value.

30. Method for spectroscopically detecting a marker comprised in the matrix according to any of claims 1 to 28, said method comprising the step of probing the marker by one or more predetermined frequencies.

31. The beaded polymer matrix according to claim 8, wherein the ratio R = a/b between a) the volume of the beaded or granulated polymer matrix and b) the average volume of the particles is in the range of from 10000000:1 to 10:1.

32. The beaded polymer matrix according to claim 31, wherein said ratio R = a/b is in the range of from 1000000:1 to 30:1.

33. The beaded polymer matrix according to claim 31, wherein said ratio R = a/b is in the range of from 1000000:1 to 100:1.

34. The beaded polymer matrix according to claim 31, wherein said ratio R = a/b is in the range of from 1000000:1 to 200:1.

35. The beaded polymer matrix according to claim 31, wherein said ratio R = a/b is in the range of from 1000000:1 to 1000:1.

36. The beaded polymer matrix according to claim 31, wherein said ratio R = a/b is in the range of from 100000:1 to 1000:1.

37. The beaded polymer matrix according to claim 31, wherein said ratio R = a/b is in the range of from 100000:1 to 2000:1.

38. The beaded or granulated polymer matrix according to claim 31-38, with an average volume of the swelled bead or granule of 0.000001 µL – 50 µL

27-09-02

39. The beaded or granulated polymer matrix according to claim 31-38, with an average volume of the swelled bead or granule of 0.00001 µL – 5 µL

40. The beaded or granulated polymer matrix according to claim 31-38, with an average volume of the swelled bead or granule of 0.001 µL – 1 µL

41. A composition comprising a plurality of different, spatially encoded, beads according to any of claims 1 to 40, wherein essentially each bead is uniquely identifiable.

42. The composition according to claim 41, wherein the composition comprises at least 10^2 uniquely identifiable beads.

43. The composition according to claim 41, wherein the composition comprises at least 10^3 uniquely identifiable beads.

44. The composition according to claim 41, wherein the composition comprises at least 10^5 uniquely identifiable beads.

45. The composition according to claim 41, wherein the composition comprises at least 10^7 uniquely identifiable beads.

46. The composition according to claim 41, wherein the composition comprises at least 10^9 uniquely identifiable beads.

47. The composition according to claim 41, wherein the composition comprises at least 10^{11} uniquely identifiable beads.

48. The composition according to claim 41, wherein the composition comprises at least 10^{13} uniquely identifiable beads.

49. The composition according to claim 41, wherein the composition comprises at least 10^{15} uniquely identifiable beads.

27-09-02

50. The composition according to claim 41, wherein the composition comprises at least 10^{17} uniquely identifiable beads.

51. The composition according to claim 41, wherein the composition comprises at least 10^{19} uniquely identifiable beads.

52. The composition according to claim 41, wherein the composition comprises at least 10^{21} uniquely identifiable beads.

53. The composition according to claim 41, wherein the composition comprises at least 10^{23} uniquely identifiable beads.

54. The composition according to any of claims 41 to 53, wherein the spatial immobilisation of the plurality of particles in each beaded polymer matrix is essentially unique for each bead.

55. The composition according to claim 54, wherein the spatial positions of particles in each bead are defined by sets of coordinates, (x,y,z) of particle centers of said particles, relative to one reference point of the detection.

56. A method of detection of relative positions in space of centers (x,y,z) of immobilized particles according to claim 1,2 and 54 where the detection is based on recording of 2D-projections of the particles.

57. A method according to claim 56 where 3 2D-projections are recorded along 3 orthogonal axis x, y and z to generate 3 sets of 2D-coordinates (y,z), (x,z) and (x,y), respectively, from which the 3D-coordinates (x,y,z) of particle centers can be derived.

58. A method according to claim 56 where a stack of 2D projections are generated by confocal or focal microscopy to recreate the 3D image matrix of the bead from which the relative particle position (x,y,z) in space can be determined.

59. A method for determination of relative particle positions within a bead according to claim 1, 2 and 54 based on focussed scanning lasers and laminar fluidics.

27-09-02

60. A method according to claim 59 in which the coordinates x and y of a particle position is determined by fast scanning two orthogonally aligned lasers over two cross sections of the moving bead while the z coordinate is determined by the time of flight of the bead at known flow rates.

61. A method according to claim 59 in which the coordinates x and y of a particle position is determined by using a single laser and a rotating mirror that via 2 or three geometrically arranged static mirrors reflects the laser beam along 2 or 3 orthogonal axis

62. A method for generating a beaded polymer matrix according to any of claims 1 to 40, said method comprising the steps of

a) synthesizing a monomer or macromonomer and a crosslinker for polymerization, and
b) mixing these with the encoding particles to give an even dispersion of particles in the mixture, and
c) polymerizing the polymer by either i) suspension polymerisation and/or; ii) inverse suspension polymerisation and/or iii) bulk polymerisation followed by granulation and/or iv) droplet polymerisation.

63. The method of claim 62, wherein the polymerisation reaction is a radical initiated chain polymerisation reaction.

64. The method of claim 62, wherein the polymerisation reaction is an anion initiated ring opening polymerisation reaction.

65. The method of claim 62, wherein the polymerisation reaction is an cation initiated ring opening polymerisation reaction.

66. A method for generating a composition according to any of claims 41 to 55 comprising a plurality of different, spatially encoded, beads, wherein essentially each beaded polymer matrix is uniquely identifiable, said method comprising the steps of

a) immobilizing particles in polymer beads or granulates.

b) isolating appropriately labelled beads or granules by automated sorting.

c) recording and storing the distance matrix for each bead or granule.

d) stepwise synthesis of molecules on functional groups of the encoded beads or granules.

e) recording the identity of each bead or granule that enter each reaction.

f) isolating beads or granules of interest in an assay or a diagnostic screen.

g) identifying the compound attached by recording the identity of each of the isolated beads or granules and comparing with the distance matrix of all beads or granules.

67. A method for identifying at least one uniquely identifiable, spatially encoded, beaded polymer matrix in a composition according to any of claims 41 to 55, said method comprising the steps of

ii) determining the unique, spatial immobilisation of a plurality of particles in the at least one bead to be identified, and

iii) identifying said at least one uniquely identifiable, spatially encoded beaded polymer matrix based on said unique determination of said spatially immobilised plurality of particles.

68. The method of claim 66 or 67, wherein a binding assay is performed by measuring the binding of a protein to a ligand bound to the polymer matrix.

69. The method of claim 66 or 67, wherein an assay is performed by measuring an enzyme activity on a substrate bound to the polymer matrix.

70. The method of claim 66 or 67, wherein an assay is performed by measuring enzyme inhibition of a molecule bound to the polymer matrix.

27-09-02

71. The method of claim 66 or 67, wherein an assay is performed by measuring receptor interaction with a compound bound to the polymer matrix.

72. The method of any of claims 56 to 71, wherein said plurality of particles comprise a fluorescently detectable marker.

73. The method of claim 72, wherein said fluorescently detectable marker is detectable by two photon fluorescence microscopy.

74. The method of claim 72, wherein said fluorescently detectable marker is detectable by one photon fluorescence microscopy.

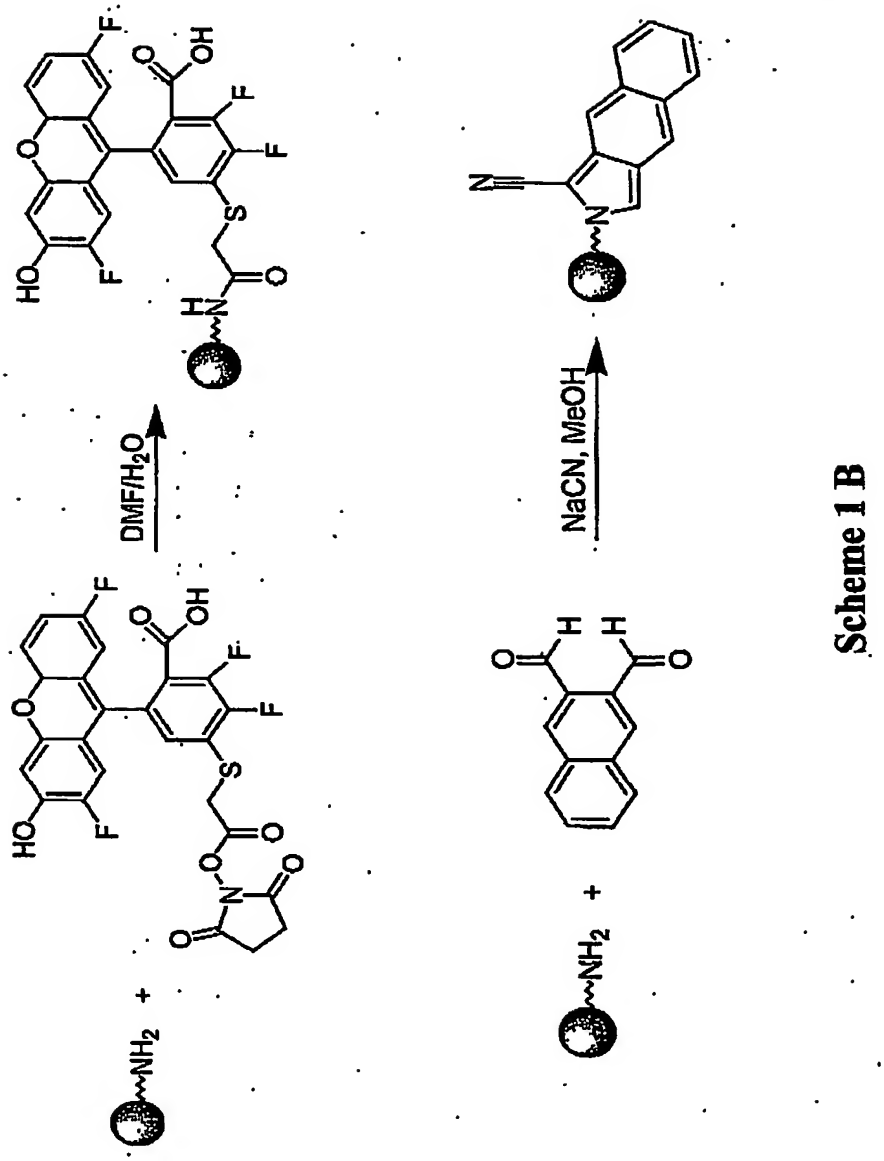
75. A beaded or granulated polymer according to any of claims 18 and 19 where the fluorescently detectable marker is selected from the group consisting of dyes based on the structure of fluorescein, oregon green, rhodamine, aminobenzoic acid, Alexa™ probes, BODIPY-dyes, cascade blue dye, coumarine, naphthalenes, dansyl, indoles, pyrenes pyridyloxazole, cascade yellow dye, Dapoxyl Dye, Fluorescamine, aromatic ortho dialdehydes, OPA and NDA, ATTO-Tag's, 7-Nitrobenz-2-Oxa-1,3-Diazole or derivatives thereof,

76. The method of claim 72, wherein said fluorescently detectable marker is a UV or visible light-Excitable Microspheres.

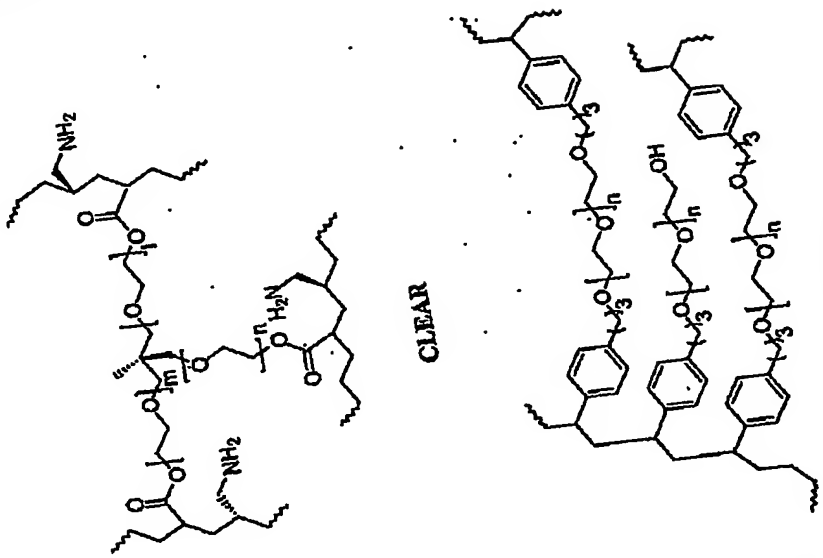
27-09-02

Labelling and immobilisation of microbeads by aqueous inverse suspension polymerization

Scheme 1 A

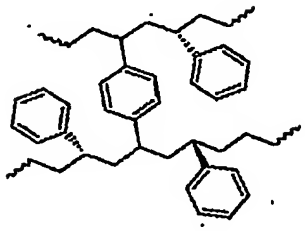


Scheme 1 B

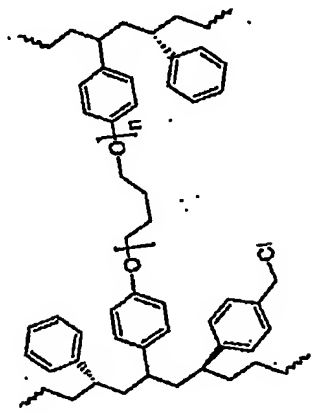


CLEAR

POEPS-3

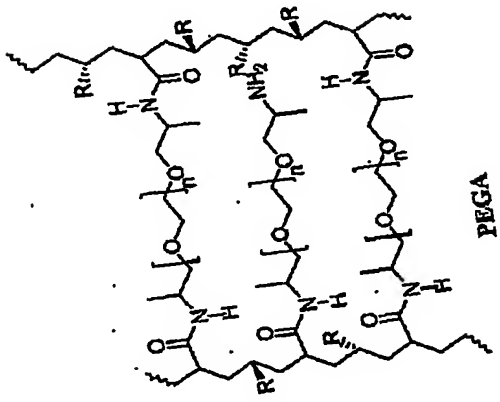


PS-DVB

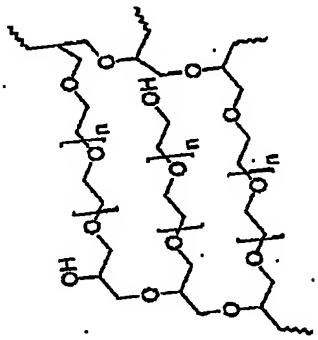


JANDAGEL

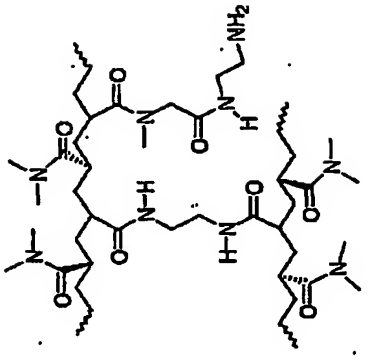
Scheme 2 A



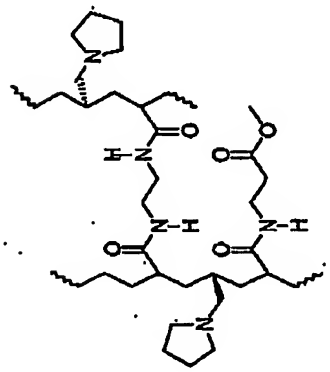
PEGA



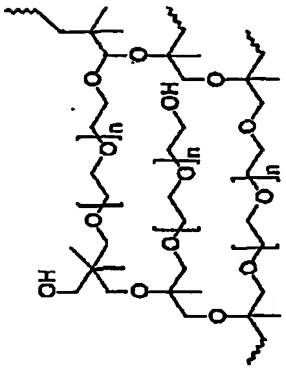
POEPOP



POLYAMIDE



EXPANSIN



SPOCC

Scheme 2 B

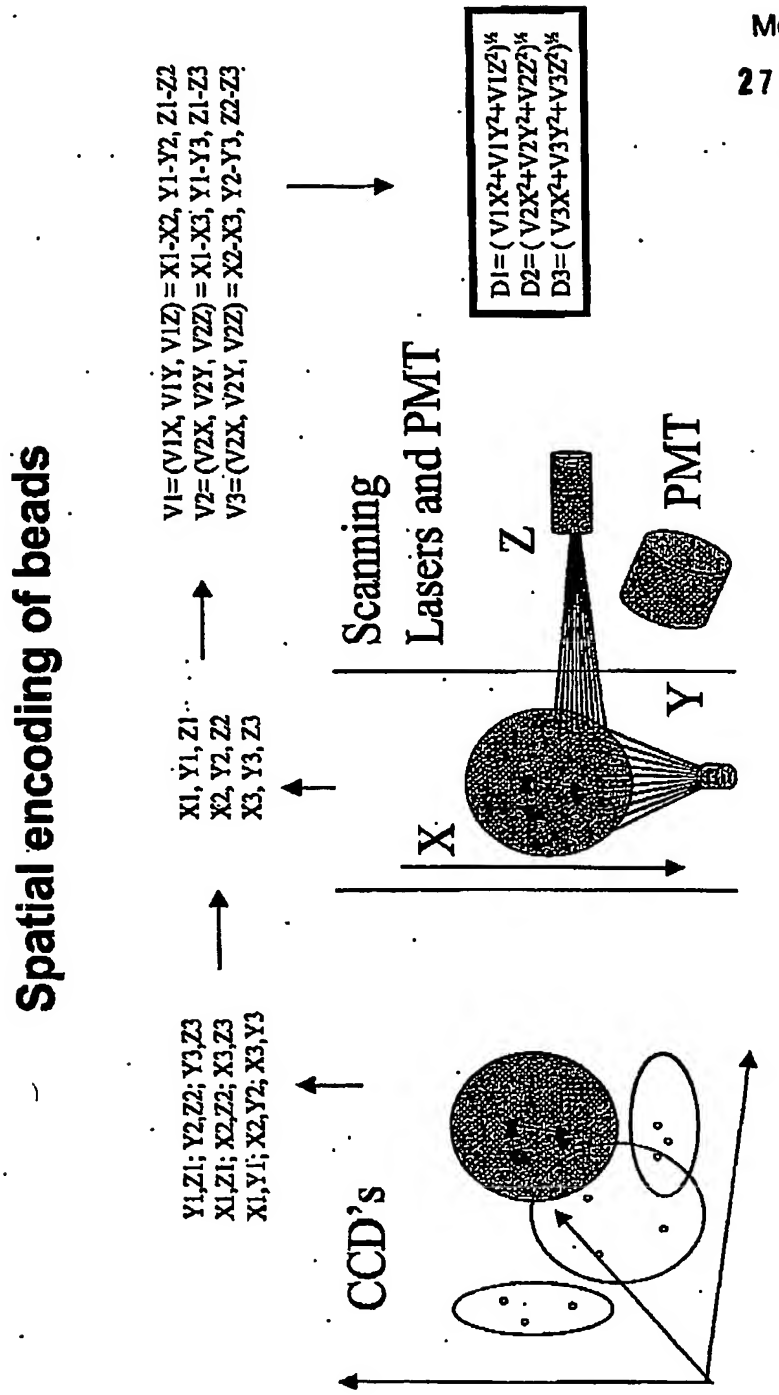
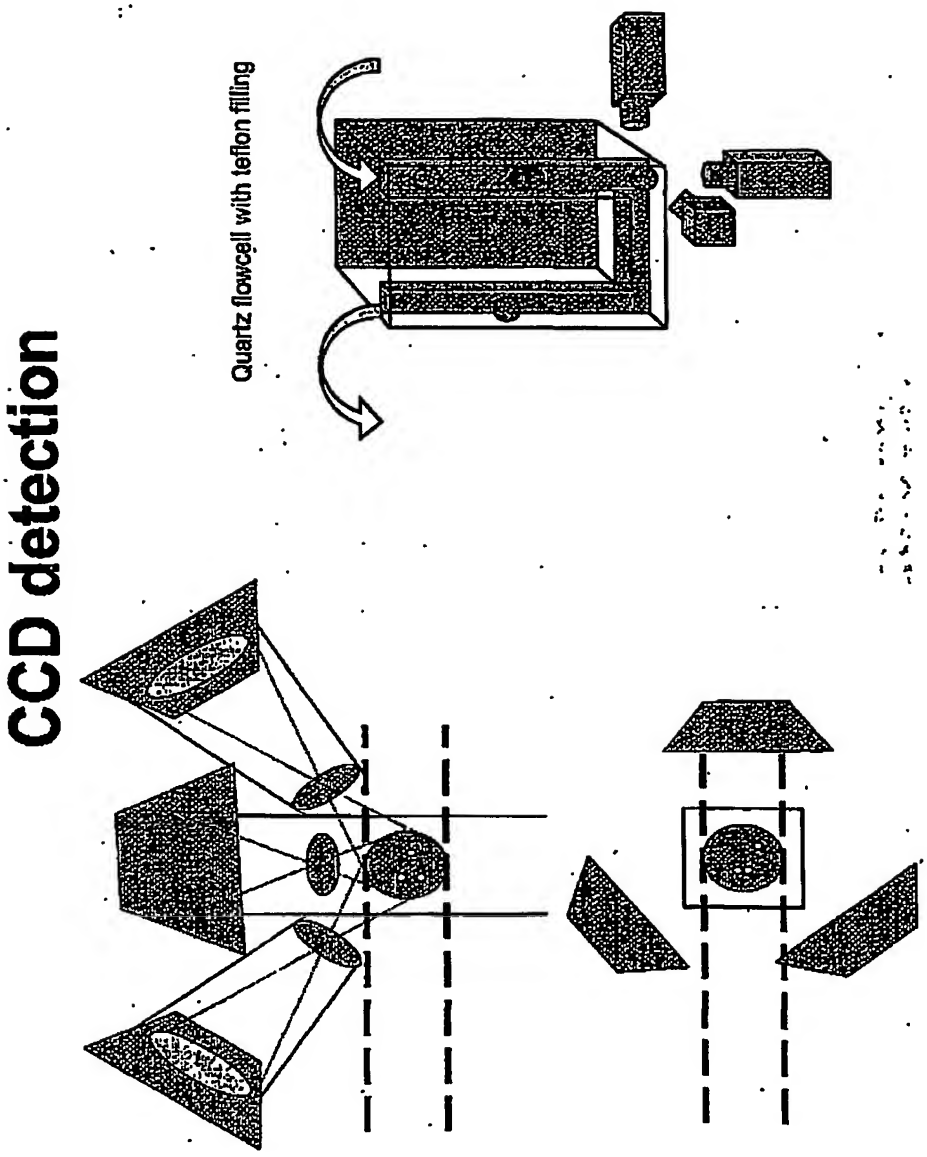


Figure 1



CCD detection system: The 3-CCD detector plates are positioned on orthogonal axis in Cartesian co-ordinate system around the flow cell

Figure 2

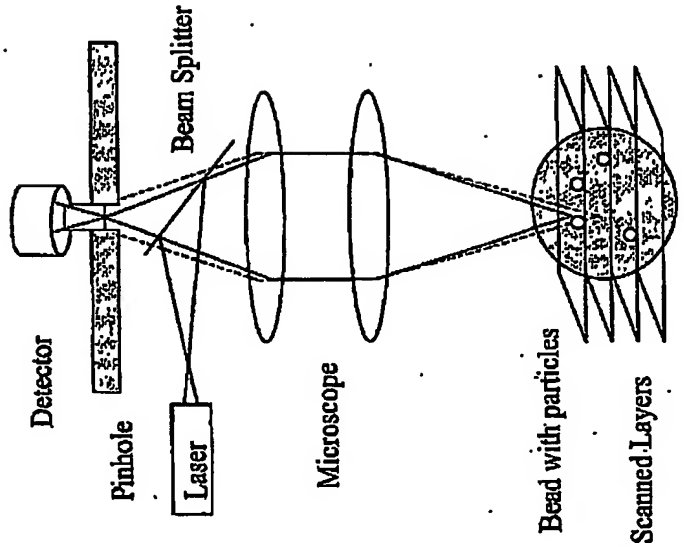


Figure 3: Recording of coordinates of particles in a bead using focal or confocal microscopy

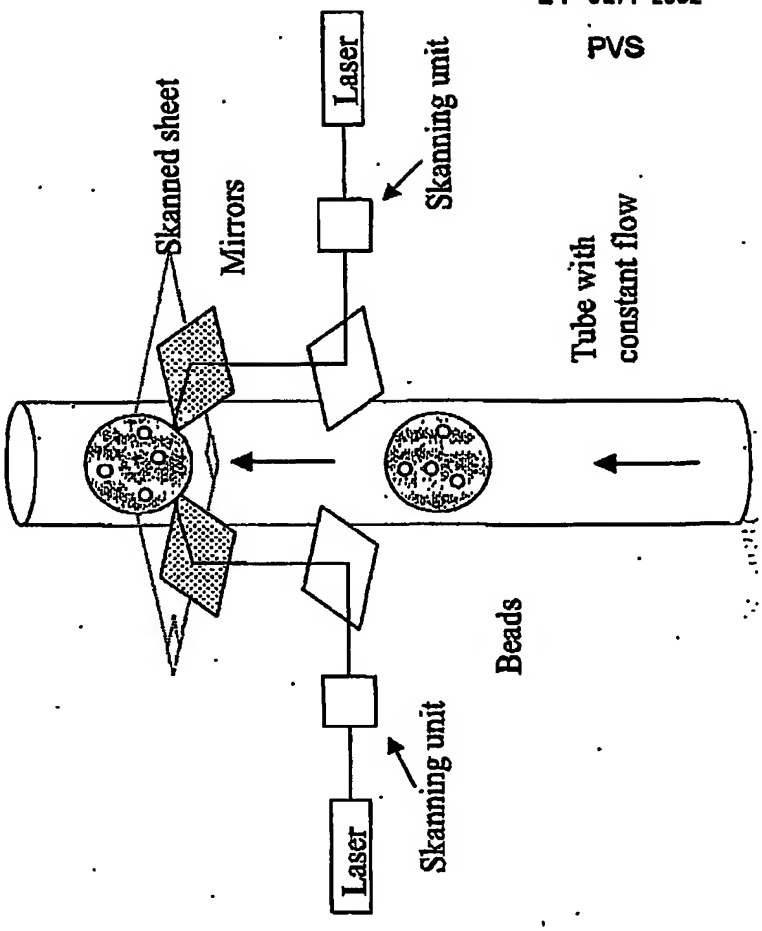


Figure 4: Recording of coordinates of particles in a moving bead by two alternating scanning lasers

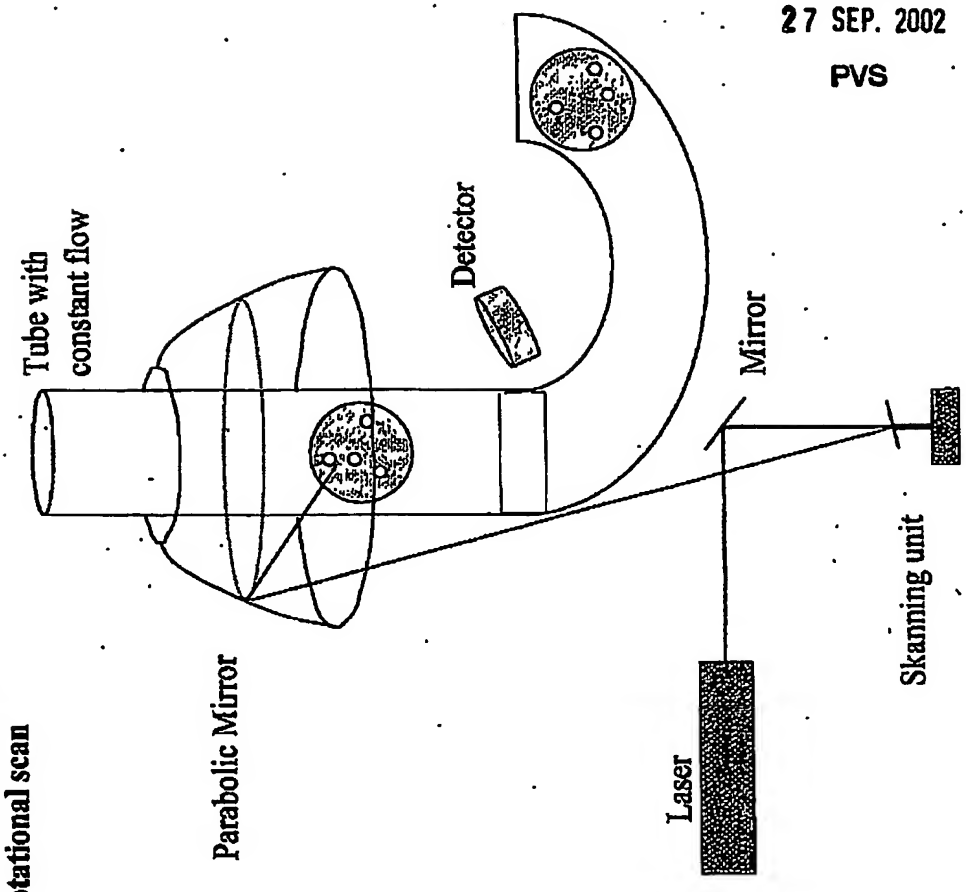


Figure 5a: Recording of coordinates of particles in a moving bead by rotational scan

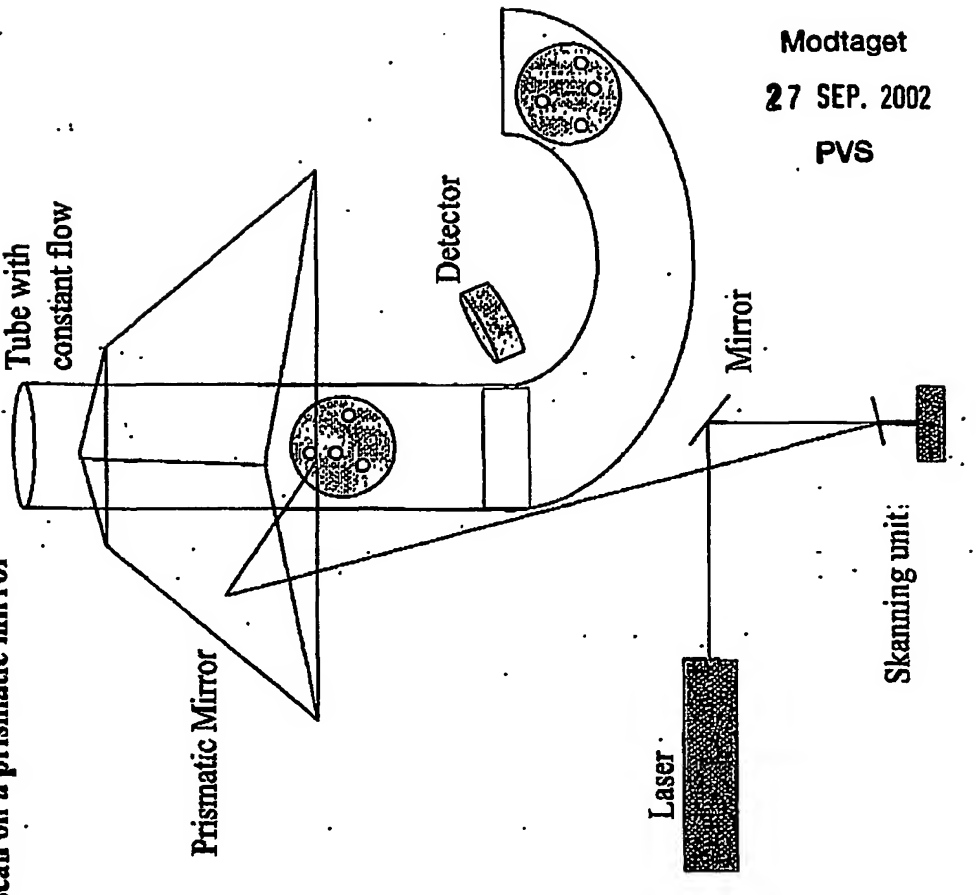
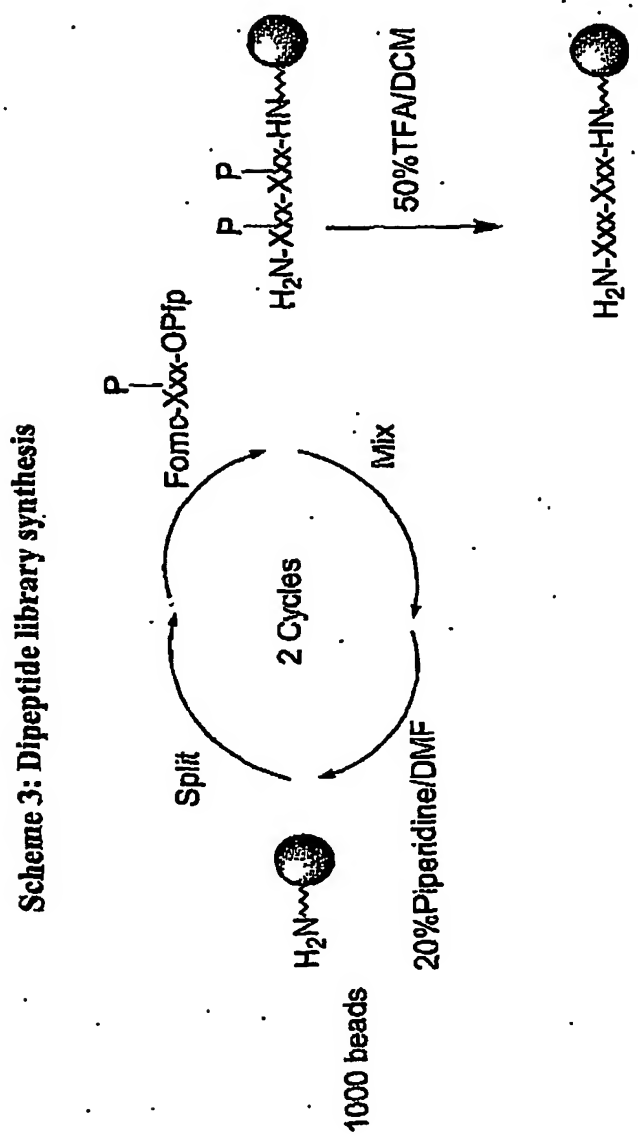


Figure 5b: Recording of coordinates of particles in a moving bead via scan on a prismatic mirror

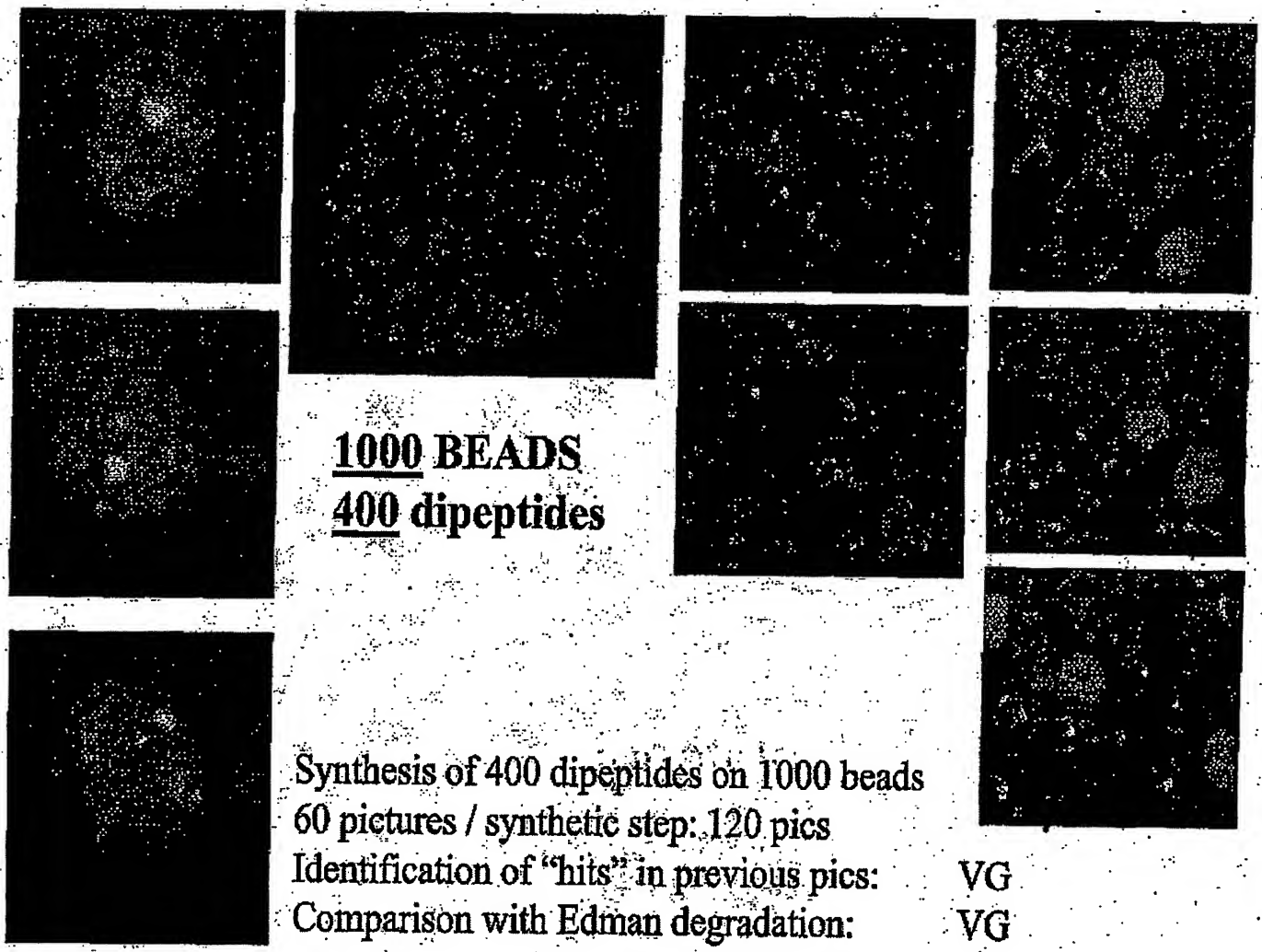


Scheme 3

PA 2002 01444

Visual decoding of dipeptide library

Figure 6 A



1000 BEADS
400 dipeptides

Synthesis of 400 dipeptides on 1000 beads
60 pictures / synthetic step: 120 pics
Identification of "hits" in previous pics: VG
Comparison with Edman degradation: VG

64

Modtaget
27 SEP. 2002
PVS

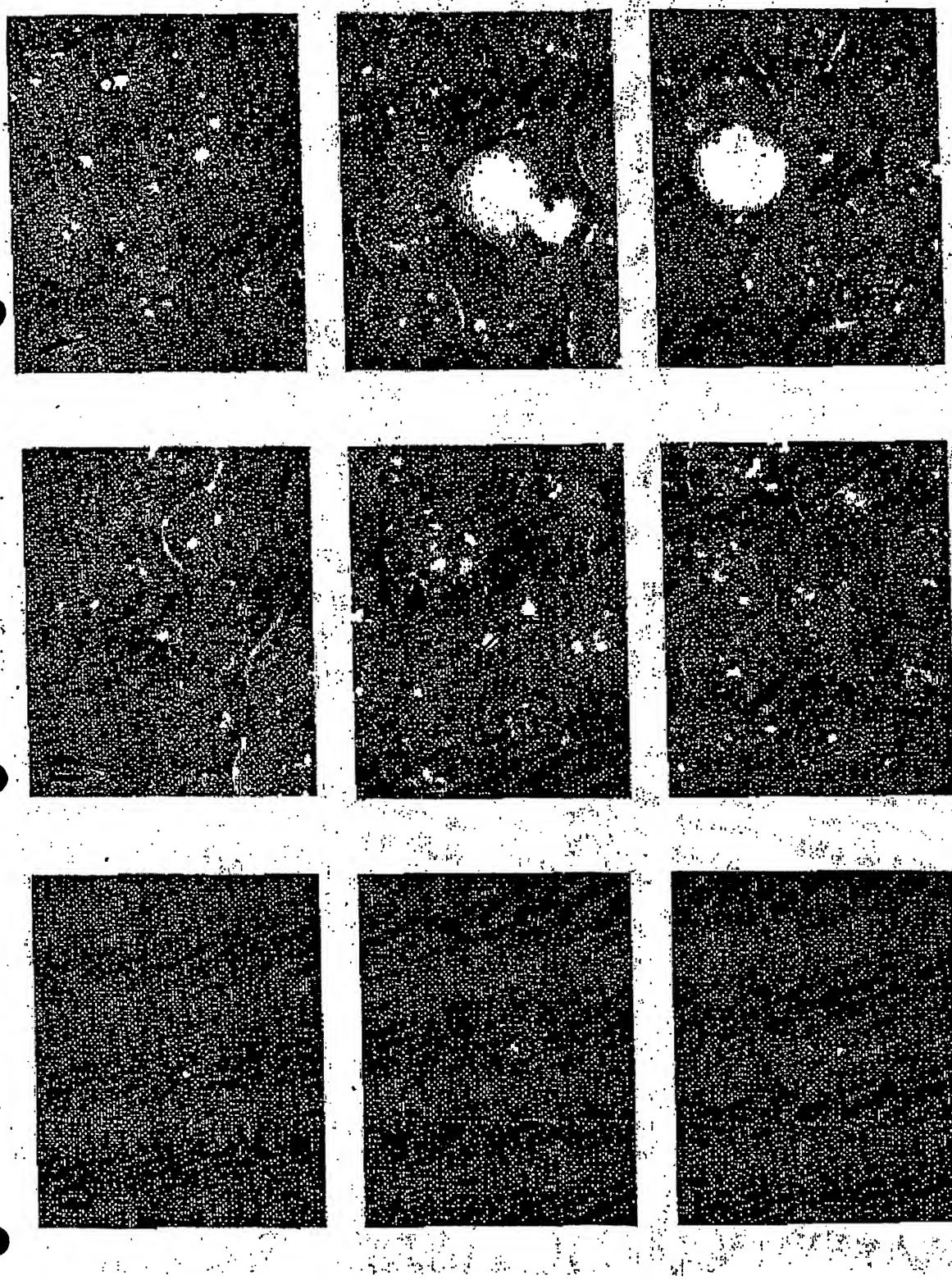


Figure 6 B

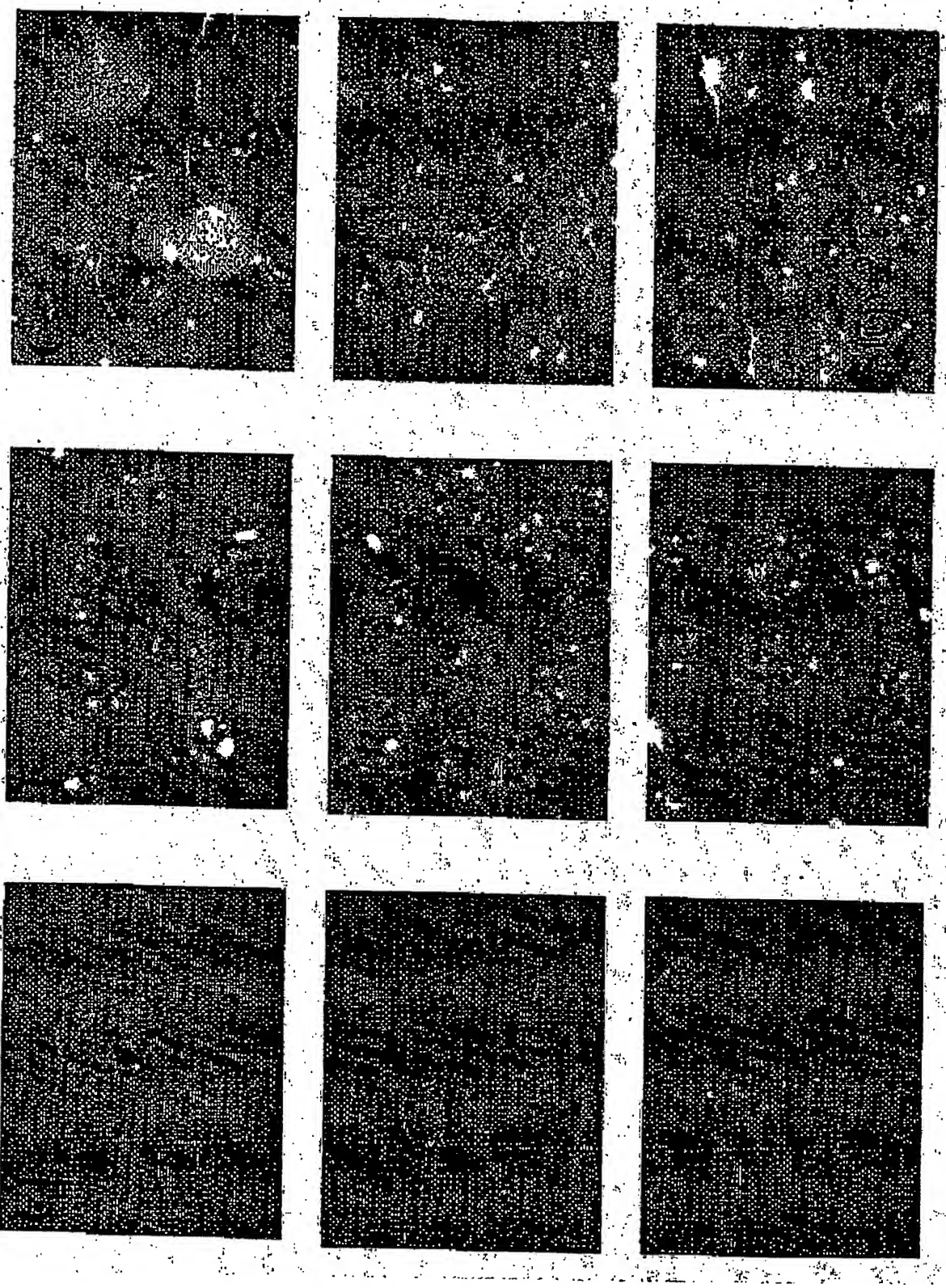


Figure 6 C

Modtaget

27 SEP. 2002

PVS

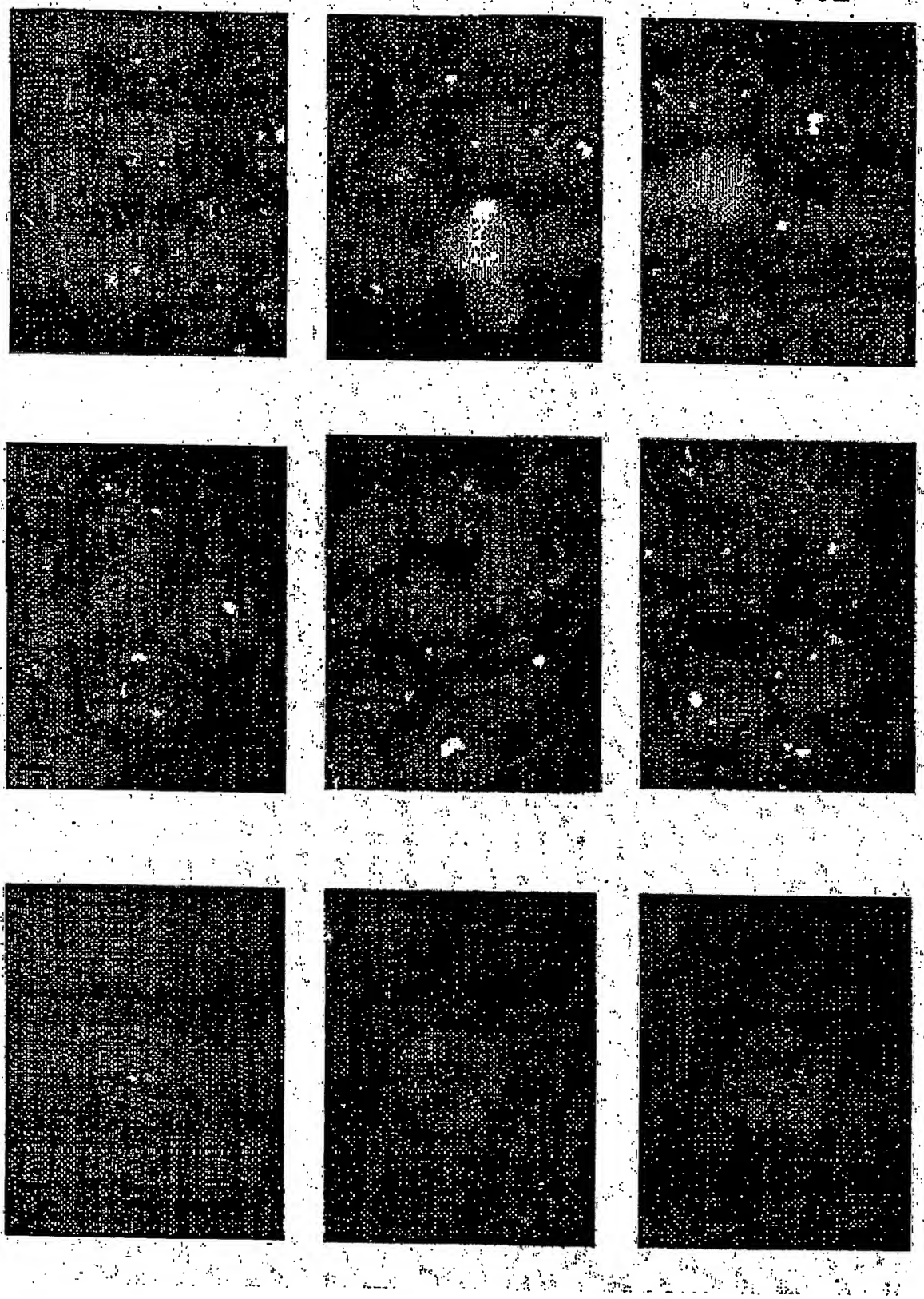


Figure 6 D

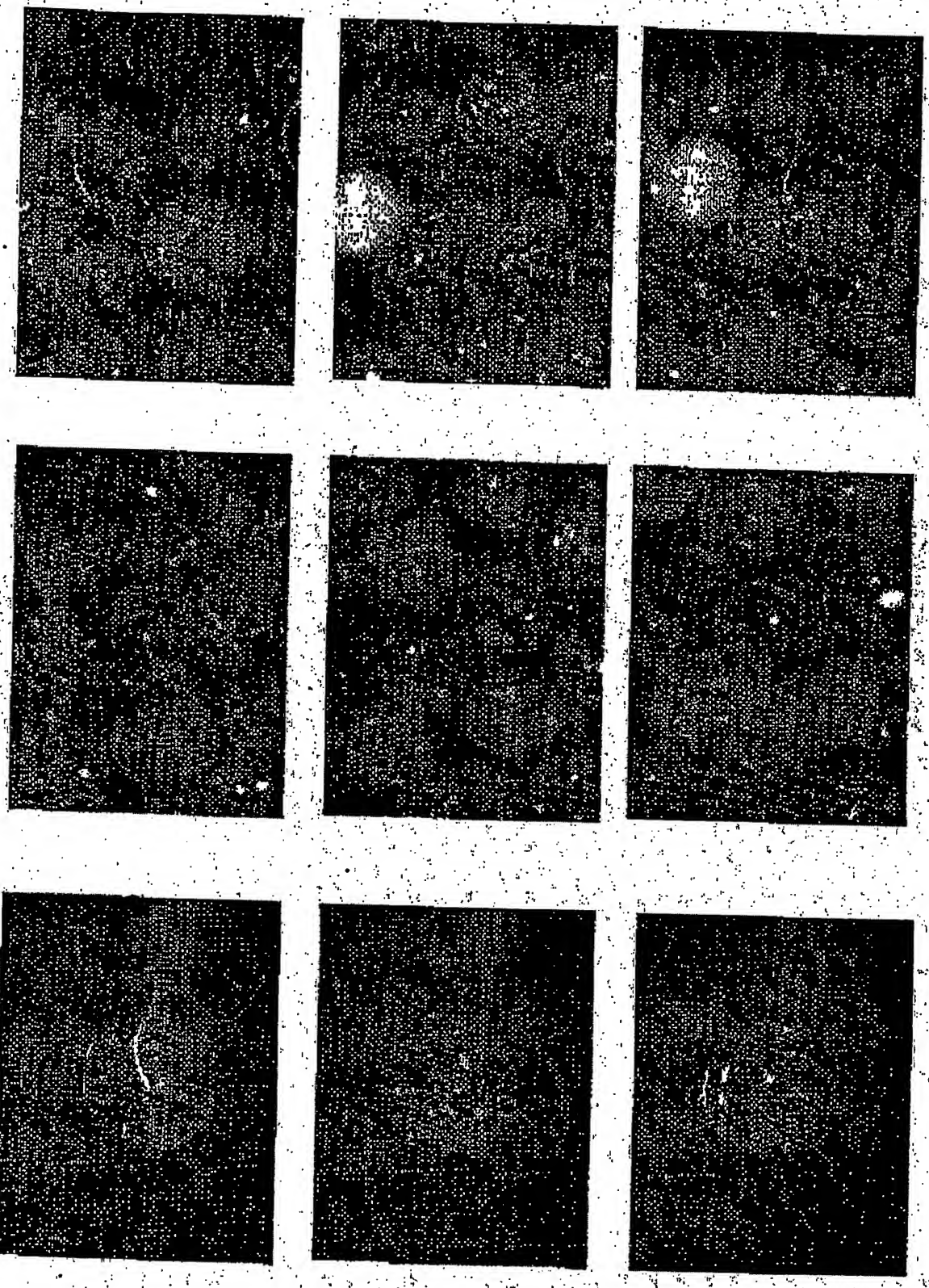


Figure 6 E

Modtaget

27 SEP. 2002

PVS

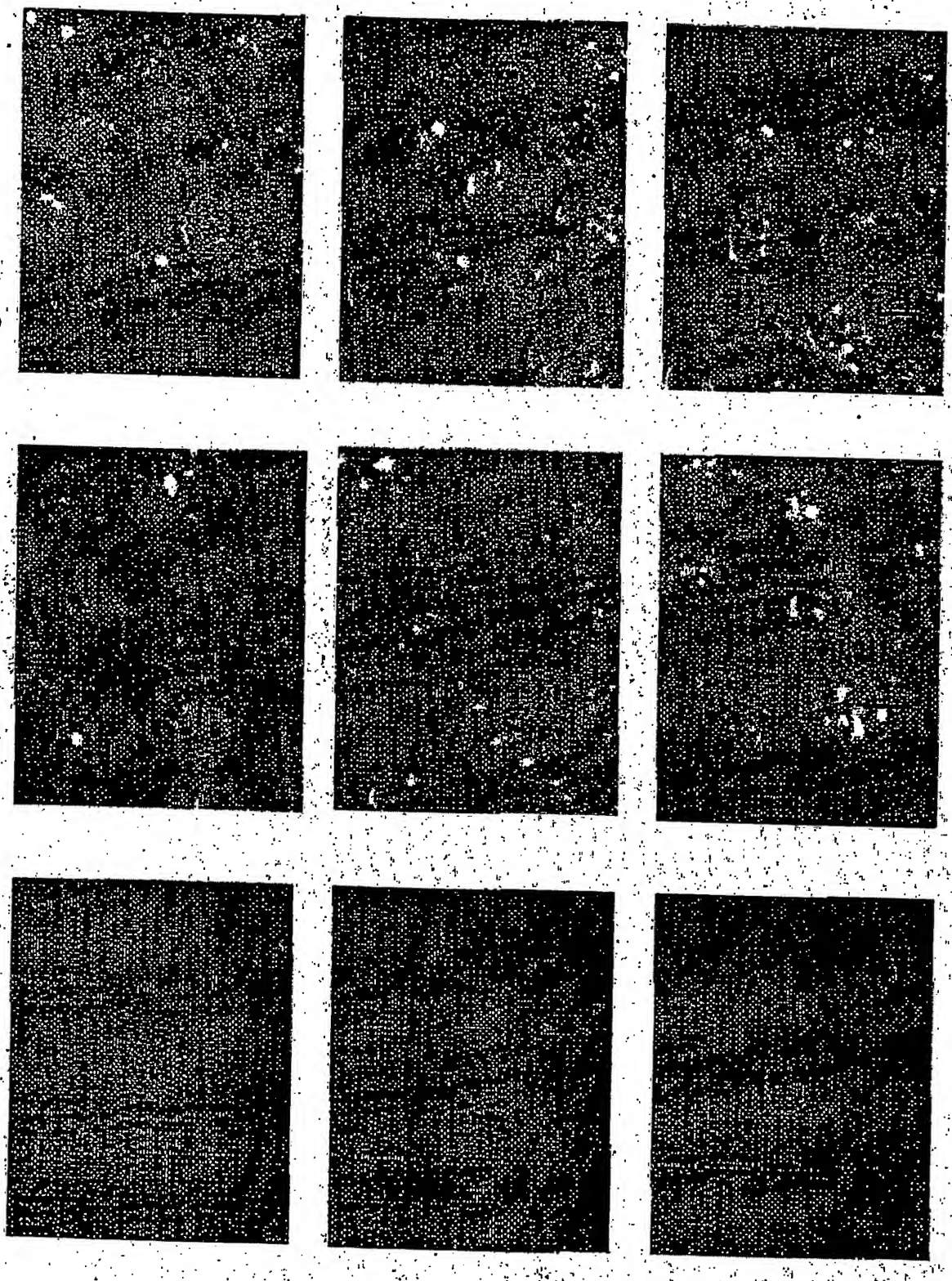


Figure 6 F

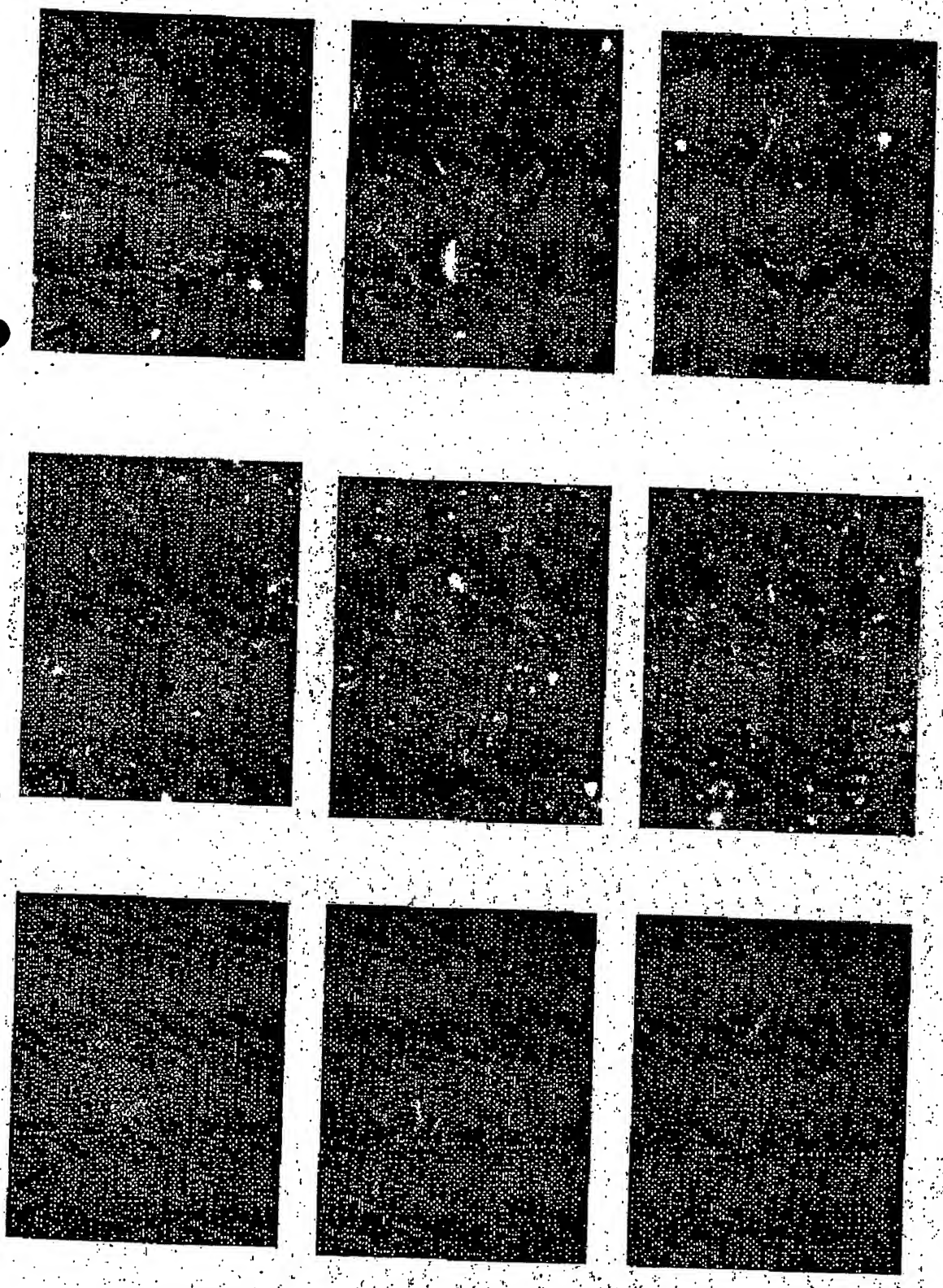


Figure 6 G

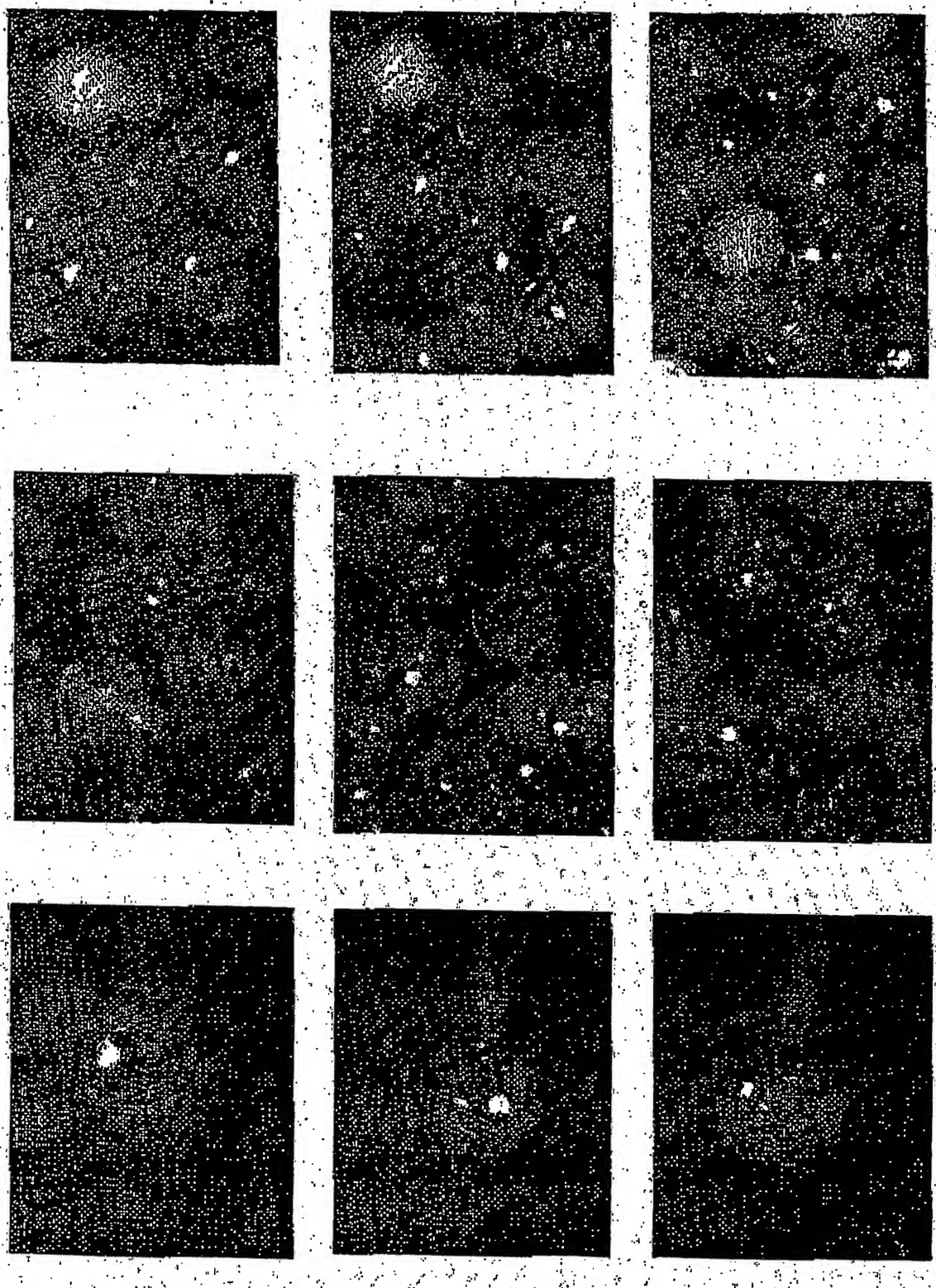


Figure 6 H

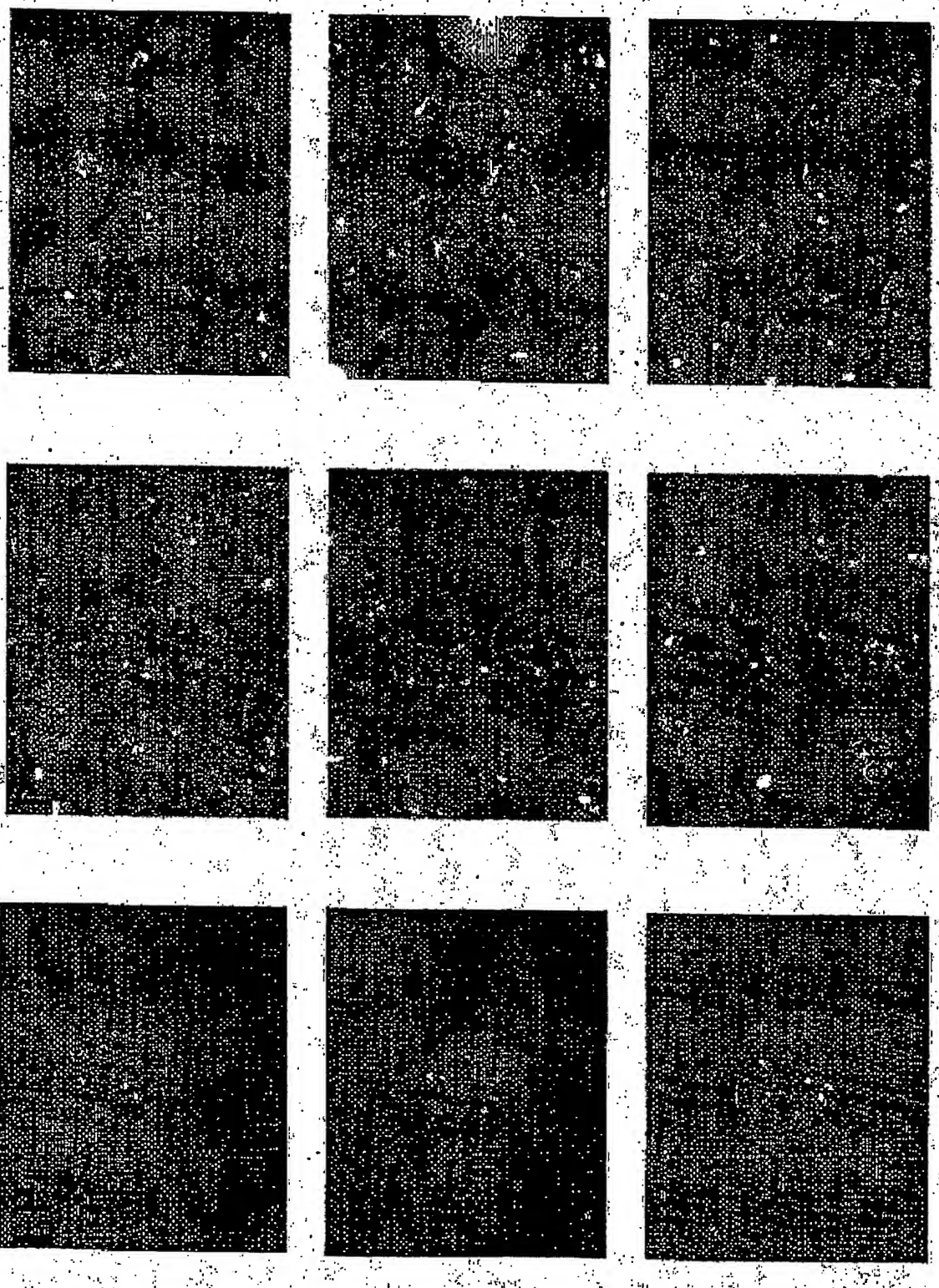


Figure 61

Table 1

Visual decoding of 20 beads from a library containing 400 dipeptides

	Seq	Pic	Other possible			Seq	Pic	Other possible
1	CF	CF			11	HF	HL	
2	AT	AY			12	NT	NT	
3	DV	DV			13	AN	AN	
4	EV	EF			14	PH	PF	
5	FR	FD			15	PK	PK	
6	GM	GM	GF		16	FR	FR	FH VR
7	IV	IV	IP		17	VG	VG	
8	LP	LP			18	RQ	RQ	VQ
9	WG	WW			19	VG	VG	
10	YW	YW			20	AL	AL	VL AI

Figure 7 A

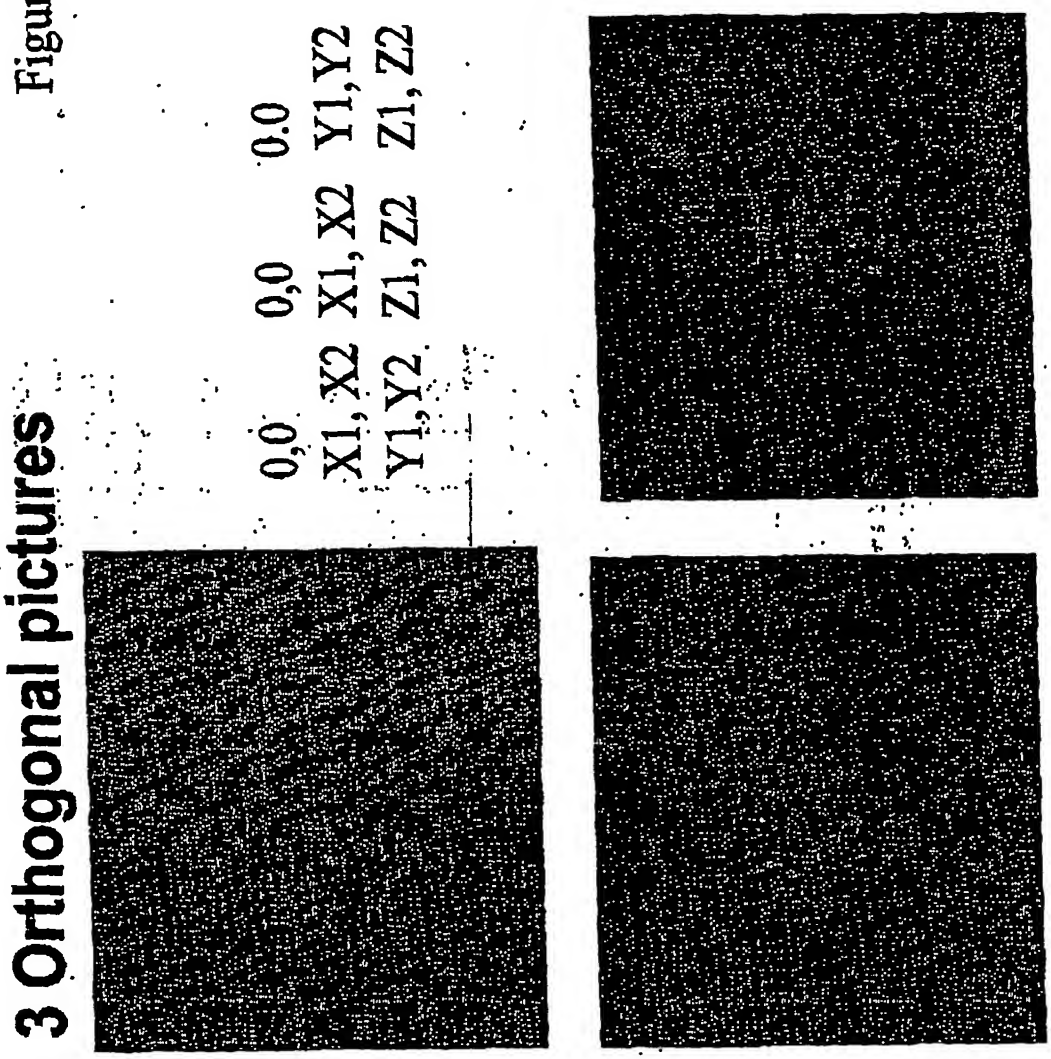
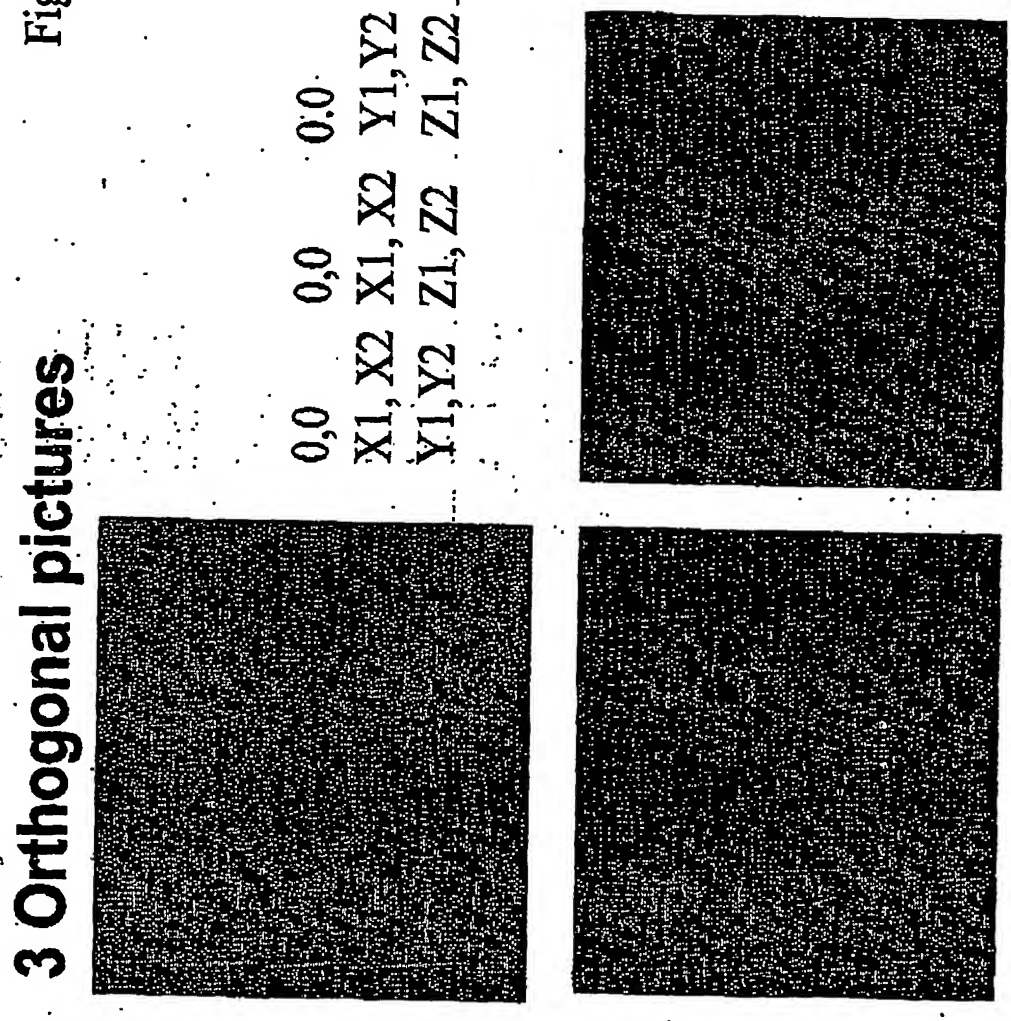


Figure 7 B



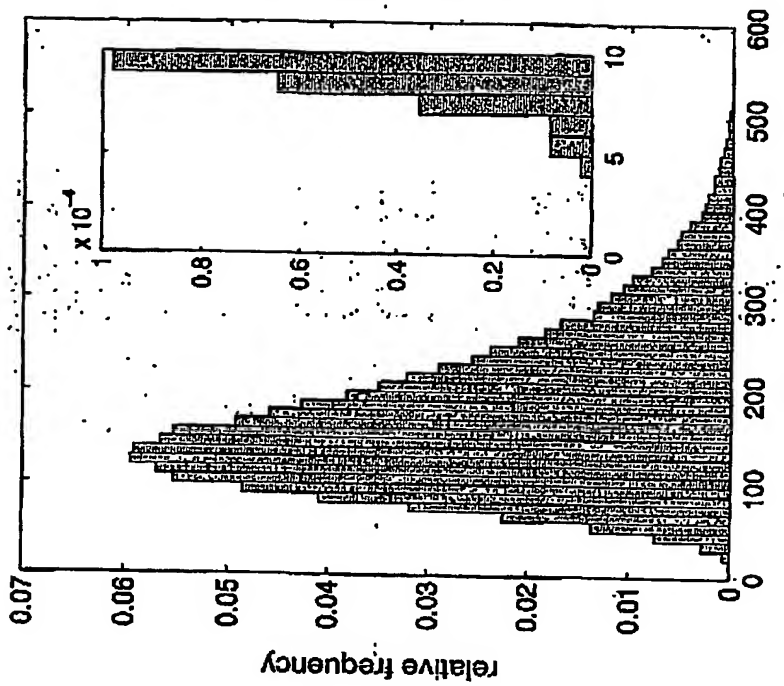


Figure 8